

5 February 1981

Two views of the causes of cancers

What is the cause of cancer? This question has for the past half-century engaged physicians, biologists and laymen. It is, of course misstated. There is no one kind of cancer and no single cause. Readers of *Nature* will, however, have been struck by the contrast between the two substantial articles on the subject published in the past few weeks. Three weeks ago (*Nature* 289, 127–130; 1981), Epstein and Swartz, under the title “Fallacies of lifestyle cancer theories”, set out to demolish the earlier arguments of Peto (*Nature* 284, 297; 1980), who had himself complained about Epstein’s book *The Politics of Cancer*. The issue in dispute is the relative weight to be given to voluntary and involuntary sources of chemical pollution in the causation of human cancer, but there are also subsidiary disputes about questions such as the proper interpretation of the epidemiological data. Is there now an epidemic of cancer, or is it merely that more people are dying of cancer because there are fewer competing (or masking) causes of death? The second article, by Cairns (*Nature* 289, 353–357), tackled almost the same question in a very different way. His objective was not to demonstrate causes but to point out the weaknesses of the mechanisms suggested in recent years as plausible explanations of how some kinds of cancers are caused. The truth, says Cairns, cannot be as simple as we would like to think. And much of what is said about the origin of cancer must be wrong.

How can these very different points of view and styles of argument be reconciled? Does it matter if they are not? And how should people, not simply specialists, approach the question of what causes cancer? Cairns’s argument deserves the most careful reading, chiefly because it shows how intricate the question is. The biology of cancer is not fully explained by the now familiar model in which (the story goes) damage to a DNA strand in the chromosome of some somatic cell can, if the damage is appropriately placed, give rise to a clone of cancerous cells. The model has the virtue that it accommodates very different carcinogens — particle radiation and chemical carcinogens, for example. Fast particles can obviously damage DNA molecules physically. The metabolites of at least some chemical carcinogens include materials likely to react with DNA, with consequences that are plausibly similar. So such events may be first causes, in Hume’s sense, of some kinds of cancer.

The model is useful, and should not be discarded. But it is also incomplete. It does not, for example, account for the length of the induction period for most kinds of cancer. If damage at a single point along a DNA strand can be a sufficient cause of cancer, why does not the cell thus affected instantly become the founding member of a cancerous clone of cells? And why is it that (as Cairns points out) such chromosomes of cancerous cells as can be seen are most often grossly abnormal in ways indicative of drastic rearrangement, not of a defect at a single point? The simple model of cancer causation can be elaborated to accommodate uncomfortable observations such as these. Conceivably, the induction period and the eventual appearance of gross chromosome abnormalities are both consequences of the need that a potentially cancerous clone of cells should evolve into a form no longer subject to the unknown restraints that normally inhibit competition between tissues within the same organism. Damaging a single point along the length of a DNA molecule may still be a sufficient cause of cancer. The chances are, however, that it is not. How else is it possible to account for the well-founded evidence that people differ genetically in their susceptibility to some kinds of cancer? And what, in any case, is to be made of

Cairns’s argument about the inherited disease xeroderma pigmentosum? The underlying trouble is a defect of the normal biochemical mechanism for repairing damaged DNA molecules. The observation that people afflicted with this genetic disease are prone to skin cancer and melanoma is consistent with the simple model. Ultraviolet light is a well known cause of damage to DNA molecules. But why are not the same people also more susceptible to other kinds of cancer caused by other carcinogenic agents? Cairns asks the question. Nobody knows the answer.

The implication of Cairns’s arguments is clear and also depressingly familiar. The phenomenon of human cancer is a problem in biology which is not yet understood. It will be time enough to talk about causes when mechanisms have been worked out. That will require not merely more of the molecular biology and cell biology now supported by the cancer research agencies but also a better understanding of the processes of differentiation, the physiology of homeostasis and even of some long-standing issues in old-fashioned biology. The prospect is nevertheless far from hopeless and, as in other fields, there is no reason why the development of better means of treating cancer should wait on a full understanding of the mechanisms. But, for the time being, the causes of cancer in Cairns’s sense are obscure.

How then can Epstein and Swartz pretend such confidence about the causes of cancer? Their starting point is that a cancer requires exposure to some carcinogenic agent, perhaps a chemical in the environment or some component of tobacco smoke. Their methods are epidemiological but not necessarily suspect on that account. Statistical analysis can establish relationships between causes and effects. Epidemiology has, for example, demonstrated the occupational hazards of vinyl chloride monomer. It is also now to be taken as a fact that people who smoke cigarettes are more likely than others to develop lung cancer. The habit of smoking cigarettes is thus in some sense a cause of lung cancer. The link is clear enough for individuals prudently not to smoke cigarettes and for governments to encourage abstinence. Yet the habit of smoking is not a first cause of lung cancer. That distinction no doubt lies with a group of chemicals not yet conclusively demonstrated. The mechanism by which lung cancer develops is not known, with the result that there is even room for argument about the influence of unknown genetic factors in the development of the disease. Such difficulties do not of course vitiate the epidemiological approach to cancer. On the contrary, there is undoubtedly great benefit to be won, both in the recognition of specific hazards and in the wider understanding of the natural history of cancer. Prudent practitioners are, however, careful not to make too much of the associations they find between effects and their supposed causes.

Epstein and Swartz appear to be more than a little cavalier in their regard for that simple rule. The trouble seems to start from *The Politics of Cancer*, whose purpose was to argue that governments (but especially the United States government) should do more to regulate occupational and environmental carcinogens, and which has set the tone for much of the discussion that has followed. The purpose, of course, is entirely legitimate. It is the method that is suspect. Epstein, writing for a general audience, has assembled an impressive list of case studies of chemicals whose use has been implicated in cancer, accurately or otherwise. The more gentle among his readers have no doubt by now been persuaded that there is no hope of avoiding cancer while they continue to live in advanced societies such as America’s. Some of them, perhaps for other reasons, will no doubt have



embraced the conclusion that the chemical industry, by the scale of its recently increased manufacture of organic chemicals, has become the chief hazard. Yet many of the items in Epstein's indictment are, to say the least, disputable — the carcinogenicity of saccharin, for example, as Peto quite properly pointed out nearly a year ago. Epstein's comment on this point (*Nature* 289, 115; 1981) implicitly confirms that view. The nature of his conclusion, and the clarity with which it is stated, seem entirely at odds with the simple rule that epidemiologists should acknowledge the possibility that their statistical association may be wrong. Self-doubt is not merely seemly but persuasive.

But is not an appearance of self-doubt inappropriate in a book whose purpose is polemical? One objective of *The Politics of Cancer* is after all to persuade governments to change their ways.

Epstein, who appears from his bibliography to be a frequent witness before congressional committees, does not lack opportunities for putting his point of view. On this occasion, however, the result may have been so to scare such a large number of readers of the book that they in turn will put pressure on their congressmen, forcing a change of policy towards the chemical industry and its products born not of reason but of fright. There can be no circumstances in which it is permissible for professional scientists to hazard the reputations of their subjects in such a cause. The overstatement of a plausible case is not permissible. To conduct scientific arguments in immoderate language is bad for the public reputation of science. This is why Cairns's way of looking at the problem, with all its inconclusiveness, is so much to be welcomed.

University empire confronts a crisis

What on earth is to be done about the University of London, which is not so much a university as a relic of a university empire? It is a huge and ungovernable federation of more than forty titularly autonomous institutions, more than half of them concerned with medical education. There are eight non-medical colleges providing a general undergraduate curriculum and a further ten with more specialized interests, some only at the graduate level. The schools differ greatly in size, strength and reputation. Some, such as the London School of Economics and Imperial College, are international centres of scholarship — and Imperial College is financed separately from the rest of the university on the grounds of its national importance. Others, not always through their own fault, are at the other end of the spectrum of academic excellence. In 1979–80, there were 30,000 undergraduates and 10,000 graduate students, 18 per cent of them from overseas. London's financial prospects are thus bleaker than those of other British universities because its proportion of overseas students is greater; it stands to be hit harder by the government's insistence that overseas students must now be charged full economic fees (see *Nature*, 29 January). The university has long since abandoned its role as a provider of school-leaving examinations for most of the British Commonwealth and has even, more recently, given up the invidious role of providing other British institutions of higher education with certificates of good housekeeping. Now, like the British Empire before it, the university is faced with the prospect of collapse or, at least, of rapid contraction.

How is the problem being tackled? London's first step was predictable — setting up a committee. There is the obligatory outside chairman (Sir Peter Swinnerton-Dyer, vice-chancellor of the University of Cambridge) and marching orders to suggest how resources should be redistributed so as to keep the university in being ("academic excellence" etc.) at less cost ("having regard to the need to make financial economies" etc.). The committee, which is concerned only with the non-medical parts of the university, has sought to avoid the ructions that greeted the report of the Flowers committee on medical teaching last year by publishing last week an interim discussion document. The result is a collection of figures for the costs of teaching students in different subjects in the various colleges, a solemn warning that the years ahead will see income fall by 15 per cent and student numbers by 10 per cent, and hardly a hint of the recipe for reorganization the committee has promised by the end of the year. The document is thus not so much an interim report as an incitement of the lobbying and horse-dealing that will occupy the University of London for months to come.

That something, almost anything, must be done is crystal clear. For decades, the university has been almost as much concerned with attempts to reform itself as with the teaching of students. The practical difficulty is that change is usually unacceptable to the institutions most affected, which within the federal structure of the university have the final say. The Swinnerton-Dyer committee will succeed where others have failed only if it can persuade the

constituent institutions of the university to bury their separate interests for the sake of the university to which they belong. The weakness of the interim document is that it seems designed to frighten the separate colleges into compliance with some unknown grand design by the threat of penury. The document thus urges that the university and its colleges should urgently consider how to manage redundancies among academic and other members of staff: tenure is plainly no longer sacred. It makes little of the threat, uppermost in the minds of many colleges in the past few months, that some of them may be closed or merged with others; there is merely a statement that the university can distribute as it thinks fit the funds it receives from the University Grants Committee (subject only to what the senate and the court of the university have to say). The strongest hint of what is to come is that university teachers might with advantage teach in more than one college. These possibilities, sensible enough, do not amount to a recipe for a stronger university. And they smack more of the stick than the carrot.

For the sake of the university, the Swinnerton-Dyer committee must find a more positive case to put a year from now. Although the interim document is full mostly of figures, it does acknowledge that in the end academic considerations will be paramount. What should these be? The committee must first count its blessings, which are various but not always obvious. The larger general colleges are comparable in size as well as quality with independent universities elsewhere in Britain. Some of the smaller colleges have a claim on public attention (and funds) for their tradition of educating less highly qualified entrants. Birkbeck College is the only institution of its kind in Britain with the objective of providing part-time students with an undergraduate education. Five of the independent schools (architecture, agriculture, education, pharmacy and veterinary science) are predominantly vocational; their place within the university calls for a frank but sympathetic appraisal. Should they all continue and, if so, should they continue to be supported from central funds as if their students were ordinary students? The risk is that the committee will be mesmerized by the reputations of the outstanding schools, letting the others go hang.

Problems of organization are also likely to obtrude. What kind of university should London become? The paradox in the past decade is that all parts of it have become more independent of the centre, which suits the larger schools well enough but makes it harder for the others to enjoy a sense of being part of a university. Nobody wants to see a revival of the dominance of bureaucracy, but the question must arise whether it is not the proper function of a university such as that in London to be a nursery for new kinds of university institutions — and whether the time has not arrived when places such as Imperial College should be encouraged to go their own way, to become autonomous in name as well as in fact. As things are, the equitable allocation of resources is unmanageable. How can the giants be denied? And how then can the pygmies enjoy a sense of partnership in a common enterprise — not that of being mere pygmies?

Cutback on West German development

Money now in short supply in Bonn

The West German research ministry is being forced by economic problems to reassess priorities. Last week, the Bundestag began a discussion of the proposals put forward by the new science minister, Andreas von Bulow, whose plan for 1981 will increase the budget by only 5.2 per cent, now less than the rate of inflation. This new small increase has come as a shock to West German planners who are unused to having their budget cut.

The increase is less than had been forecast in the three-year forward plan published a year ago, which promised an increase of 10 per cent (in money terms) for 1981. The new research budget is not, however, out of line with revisions of other government expenditures, consequences of the worsening economy.

The research ministry, which controls the lion's share of funds for German research, is planning to save money by cancelling some new projects. Mr von Bulow says that he does not want to delay projects already under way. Expensive demonstration projects are likely to suffer most, but the government hopes that some of their cost can be taken over by the utilities (in nuclear power and coal), by industry, the Länder or local government. University research may do better because of the commitment to keeping university researchers in employment. The aim is to keep the proportion of the budget spent on basic research at just over 30 per cent.

Three particular projects are affected. A demonstration track for testing an advanced passenger train and a new tokamak device for fusion experiments at the Institute for Plasma Physics in Garching have already been abandoned. The cancellation of the demonstration track is expected to save DM30–40 million this year and DM 250 million by the end of 1983. The fusion device, known as Zephyr, would have cost an estimated DM500 million to build at 1980 prices. Its cancellation has removed a major project from the work of the plasma physics institute and has led the ministry to ask the institute to become more involved in the Joint European Torus (JET) under construction in Britain.

A decision has yet to be reached on the fate of the third project, a coal liquefaction plant being built in Virginia, United States. Germany and Japan are each expected to contribute 25 per cent of its cost and the Bundestag is having to assess the repercussions of cancellation.

Research fields likely to receive more than a 5.2 per cent increase include coal, radioactive waste disposal, micro-electronics, biotechnology and polar research. The likely losers include transport, building, space research and the large nuclear research centres. University building is also to be reduced and savings will be made at the Deutsches Elektronen-Synchrotron (DESY) by reducing the operating time of PETRA, the electron-positron machine. In all fields, the aim is to cut spending on development in favour of research.

The universities will nevertheless be affected to some extent. The ministry helps to support some specific university projects even though most of the money comes from Länder and from the Deutsches

Forschungsgemeinschaft, the chief source of funds for university research. The Länder, which support the basic infrastructure including staff salaries, are also cutting increases in their budgets, but they are limited by their commitment to keep tenured staff in employment. The Forschungsgemeinschaft's budget is intended to increase from DM780 million in 1980 to DM830 million in 1981, an increase of 6.4 per cent.

In a revision of the forward plan to 1983, savings of DM2,000 million on the entire federal government research budget are planned for the next three years. The Bundestag's deliberations on the research budget will last for another few months, but a radical departure from the proposals is unlikely.

Judy Redfearn

Polish students sit in at universities

Poland now also has its student sit-ins. At the University of Lodz, 6,000 students drawn from the university, the technical university and the medical school have so far been involved in a sit-in which began in mid-January. The academic work of these establishments is virtually paralysed. The students' demand is for university autonomy and a less rigid curriculum.

Lodz is not unique. At the end of November, there was a sit-in lasting several days at the University of Warsaw, while in January some 200 final-year students of the Wrocław agricultural academy held a sit-in over issues such as the system of examinations and credits. In particular, they demanded that the present system of rigid lectures and examinations in "socio-political sciences" should be replaced by informal seminars.

The issue of "socio-political" teaching is one of the fifty-odd grievances of the Lodz protesters. The range of their complaints is considerable and, as the Minister of Science, Higher Education and Technology, Janusz Gorski, said after talks with the university senate last week, many of them are impractical. A maximum of 3 months military service for students, for example, would be meaningless. The most striking feature of the Lodz protest, however, is not the range of demands but the range of participants; the action is backed not only by the new Independent Students' Association and the Solidarity chapters of the academic staff, but also by the university party committee and the "old" organizations — the Association of Polish Socialist Students and the Union of Polish Teachers.

The Lodz protest is a symptom of discontent that runs throughout all Polish academic life. Implementation of the Gdansk Accords, signed at the end of the summer, is slow, and delays in drafting new bills on academic autonomy and the reduction of censorship to the minimum

needs of national security have evoked much anxiety that the terms of the accords may remain paper pledges only.

Thus the censorship bill, promised for 30 November, which is to take academic publishing out of the censors' control, has not yet appeared, even though the extended deadline of 15 January has been passed. Two draft bills have in fact been produced — one by the Minister of Justice, Jerzy Bafia, and one by a "public drafting committee", headed by Warsaw philosopher Dr Klemens Szaniawski. The problem of reconciling the two has become bogged down in the question of accountability. Minister Bafia would like the Office of Press, Publications and Performances (the censorship) to remain, as now, accountable to the Council of Ministers (the Cabinet). Dr Szaniawski's committee, however, wants it to be answerable to the Sejm (Parliament). Many of the proposals for "social renewal" put forward in recent months have called for the Sejm to play a more active role in the political life of the country.

In the absence of legislation, some universities have been quietly making their own reforms. Some rectors have made available to their students the university holdings of "restricted" texts.

Anomalies inevitably abound. Thus last week the Procurator General's office warned that issuing uncensored publications still carries a potentially heavy gaol sentence. Yet a few days previously, the censors had passed an issue of the Catholic weekly *Tygodnik Powszechny* which described lectures given in Gdansk by the clandestine "Society for Academic Courses" (Flying University) — lectures which, in pamphlet form, are regularly published by the underground press.

Censorship is only one of the burning academic issues. Dr Aleksander Gieysztor, the newly elected president of the Polish Academy of Sciences, sees the

democratization of academic life as a two-stage process. The first, minor, stage, he observed recently, is "already under way" — the introduction of regulations leading to "autonomy, democratization and better information". The second, and more important stage, is to reform the "overdeveloped" administration, and to ensure that scientific committees truly represent the interests of their members.

Cutbacks in the scientific bureaucracy will be greatly welcomed in the natural and technical sciences where the hierarchical classification of research projects, introduced in the early 1970s, means not only that virtually all research has to be orientated to some need of the national economy but also that there should be a vast bureaucracy to deal with funds and administration.

Given less paper-shuffling and a certain latitude in undirected research, few academics would overlook the needs of the economy. On the contrary, Solidarity has already set up its own "All-Poland Coordinating Committee for Science" and the Solidarity chapters in the various universities have put forward their own proposals for what should be done.

Some proposals are ambitious, such as that drawn up two weeks ago by the Jagellonian University of Krakow which calls for action on censorship, visas for academic travel, health service reform, pollution and the amelioration of the social conditions leading to alcoholism. Others are more specific, such as the defence issued by the Maria Curie-Skłodowska University of Lublin of the claim of Poland's 3.5 million peasant farmers to the right to form a trade union. **Vera Rich**

Telecommunications suit

Pact postponed

For lack of an assistant attorney-general, the Reagan Administration has had to postpone the settlement of its anti-trust suit against AT&T, the telephone company. Details of the settlement, which were to have been made public last Friday, will not now become known until 2 March, the date set by the trial judge, Howard H. Greene, for the submission of a settlement between the telephone company and the US Department of Justice.

The suit was begun in November 1974, and the trial opened on 16 January with formal statements by the two parties, followed by a recess. The further postponement is at the request of the Department of Justice, which explained in a letter to the court that it could not proceed until the Administration had appointed an assistant attorney-general with responsibility for anti-trust affairs.

The settlement, the essence of which has already been agreed, will have an important influence not merely on the future shape of the telecommunications industry in the United States but also on the

future role of the Bell Laboratories within it. The terms of the settlement are almost certain to include AT&T's separation of its ownership and management of the United States telecommunications network from its manufacturing subsidiary, Western Electric.

In a letter to the court supporting the request for a further postponement of the settlement, AT&T said last week that it had already bound itself to certain undisclosed conditions. There is increasing speculation that, when published on 2 March, these will be found to embody the reorganization of the corporation announced in August last year, involving separate management and accounting for the operating and manufacturing arms of the business. The future of Bell Labs, undecided then, is still in the air.

There is a sense in which the anti-trust suit by the Department of Justice has been overtaken by events, especially by the ruling of the Federal Communications Commission (see *Nature*, 11 September 1980) that companies wishing to supply terminal equipment cannot also manage the common carrier networks that connect them. This decision of what is known as the "Second Computer Inquiry" seems to have been the spur for the reorganization announced by AT&T last summer. Separately from the anti-trust suit against it, AT&T is applying to the courts to have the deadline for the reorganization decreed by the commission (1 March 1982) extended on the grounds that it cannot bring about the "biggest corporate reorganization in history" by then.

European Community

Squeezing grants

Brussels

Haggling among member states is likely to force down the increase in the budget proposed by the European Community for its scientific and technical training programme. Last Thursday, the member states' permanent representatives to the Community met for the second time and finally decided to allocate to it 8.8 million EUA (European Units of Account) or £4.5 million, which is 3 million EUA less than the original modest proposal. A final decision has still to be taken by the Council of Ministers, but this is expected within the next two weeks. Depending on how the European Community chooses to allocate grants, there will probably be the equivalent of about 340 scholarships.

While the United Kingdom was in favour of keeping the programme at its previous size of 4.5 million EUA, the French were insistent that it should be reduced. Such was their concern that the French felt unable to give their consent without calling Paris for permission. Other member countries were more worried about the number and size of the individual grants. Ireland was in favour of smaller grants for

more researchers while the Danes preferred the idea of devoting larger sums to contract researchers of a high quality.

The four-year programme is intended to give students and junior lecturers a chance to study in another part of the Community. This can be either in one of the joint research centres (Geel, Ispra, Karlsruhe or Petten) or in research institutions in another member state with whom the Commission has concluded research contracts. Those wishing to study at home will be forced to accept a cut in their grant of 25 per cent. About 320 grants were allocated in the last programme but since the new programme must include grants to Greek researchers, there will be no increase in the number available in the rest of the Community.

Normally, grants are awarded for up to a year, but they may be extended to two years for those undertaking major research projects. Part of the aim of the programme, however, is to promote contracts between those responsible for the implementation of research programmes and the relevant education and training establishments. Details can be had from the Directorate-General for Research, Science and Education, Rue de la Loi, Brussels 1049.

Last week's meeting also decided on the budget for the next four-year research programme on the environment. The Commission's initial proposal of 51 million EUA was reduced to 42 million EUA. The programme includes the continuation of a major effort in the field of climatology and other areas covered are the treatment of sewerage sludge, air pollution and organic micropollutants in water. Here again, France was keen to see the budget cut, in this case to 35 million EUA. The Greek delegate spoke for seismological studies, which won agreement in principle. Formal research programmes are at present being examined in the Council of Ministers. **Jasper Becker**

UN energy conference

Prospects improve

Washington

Prospects are looking up for the forthcoming United Nations Conference on New and Renewable Sources of Energy, due to take place in Nairobi, Kenya, in August.

Last summer, the second meeting of the conference's preparatory committee found the secretariat's efforts so far behind schedule that postponement was seriously considered. In addition, the Kenyan government began to develop cold feet about the costs of staging a major international conference, the first since the 1979 Vienna meeting on science and technology for development, and to mutter about withdrawing its invitation.

But neither threat has materialized. And when a group meets in New York at the end of this month to process the reports of eight

technical panels on the prospects for different energy sources, it is expected to be in a spirit of optimism that few thought possible six months ago.

One reason is the recent appointment of a new secretary general for the conference, Mr Enrique Iglesias, a Uruguayan who is also executive secretary of the Economic Commission for Latin America. Mr Iglesias, thought to be a candidate for Mr Kurt Waldheim's job as Secretary General of the United Nations, succeeds Mr Mohamed H. Gherab, who has recently been charged with accepting loans from subordinate officials.

A second reason for guarded optimism about the energy conference is that at present the UN secretariat seems to be successfully treading the delicate line between the technical and the political.

It has now been generally accepted that, given a lack of time and resources, the conference can only achieve a limited "state of the art" review of new and renewable energy sources (including solar, geothermal, wind, tidal, biomass, oil shale and hydropower energy). The main focus is therefore likely to be on the institutional mechanisms that can accelerate research and development on these various energy sources — and the obstacles that stand in the way of their implementation.

It is unlikely, however, that any new institution will emerge from the Nairobi conference. Proposals are more likely to be along the lines of a scheme being worked on by the World Bank to form an international network of energy research centres, with a clearly defined set of global priorities distributed in a way that minimizes duplication of research efforts.

Parallel initiatives are also under way inside the United Nations Development Programme (UNDP), which last summer set up a new energy account. UNDP has already received \$3.5 million from the World Bank to carry out a survey of the energy needs of 60 developing countries, as part of the ambitious scheme announced by bank president Robert McNamara last year for a programme of energy loans and investment to total about \$25,000 million by 1985.

The main concern of the UN conference organizers is to devise a set of policy proposals that will be sufficiently specific to meet the recommendations of the technical panels, but sufficiently broad to generate the necessary political support.

One idea under discussion, for example, is a coordinated effort to replant trees that have been cut down for fuel. The World Bank has already proposed raising \$1,000 million towards such a scheme, on the basis that a comparable sum would be found by the individual countries concerned.

Inevitably, there will be points of conflict, some of which have already come to the surface. Little attention, for example, will be given to the environmental efforts of different energy sources, a problem at present of greater concern to

the developed than the developing world. Similarly, the United Nations General Assembly has explicitly stated that the conference will not consider conservation technologies, even though many in the developed countries feel that a reduction in demand is one of the likeliest ways of tackling the energy problem.

There is also dispute about the role of the oil-producing nations. These hold many of the important cards, both in terms of their ability to affect energy prices and in having the cash surpluses available to which access would be needed for any major investment schemes. Their attitude towards the conference remains ambiguous, although Mr McNamara has made it clear that the success of his proposals depends largely on their support.

Given the actual and potential disagreements on each of these topics, therefore, the success of the Nairobi conference still hangs in the balance. But some see light at the end of the tunnel, and argue that although time is getting short, elements such as the World Bank and UNDP initiatives, existing trends in foreign aid budgets and the completion of the technical reports are sufficient to allow a successful outcome.

David Dickson

Ariane space launcher Still in trouble

The problems of Ariane, Europe's hope for a space launcher, are not over yet. The third test flight, delayed after a failure in a first stage engine on the second test flight last summer, is now unlikely to get off the ground until well after June, the date to which the European Space Agency is still clinging. The difficulty is that no precise explanation of what went wrong with the second flight has yet emerged, and correcting the fault still seems to be a matter of trial and error.

A new schedule for the Ariane programme will not be released until one of two modified fuel injection systems identified as the cause of the fault has been chosen after tests expected to last 4–8 weeks.

The second test flight failed after high amplitude oscillations at 2,300 and 2,700 Hz developed in one of the first stage engines. Tests last October with modified fuel injectors of improved tolerance seemed to solve the problem at 2,300 Hz, but the 2,700 Hz oscillation remained.

Oscillations at these frequencies had not shown up in early tests of the Ariane engines and injectors. But tests up to the middle of last month indicate that the injectors used in early Ariane development differ from new ones, suggesting that they have been modified during assembly, preparation or testing. Nobody has been able to discover precisely how the injectors were modified, but the space agency says it is now trying to increase the margin of error acceptable in the injector design.

The latest line of attack is to test two modified injectors in the hope that one of them will be free from oscillation problems. Officials are hopeful that one system can be chosen within the next couple of months and work can begin on preparing the third test flight. Even if there are no more setbacks, however, a launch in June seems optimistic.

Meanwhile, the French space agency, whose idea it was that Europe should build its own space launcher, is now planning to propose another multi-million dollar venture to the European Space Agency. France wants to be in on the "industrialization" of space in the 1990s and hopes to suggest a remote controlled space laboratory, along the lines of the Russian Salyut space station.

The plan is to build a laboratory for materials processing under micro-gravity that would be placed in geosynchronous orbit by Ariane. The laboratory would be serviced by an expendable vehicle that would deliver supplies and bring processed materials back to earth. A third spacecraft, also in orbit, would be capable of building large structures and experiments for use by the laboratory. The proposal is still only a feasibility study, and will not be put to the space agency before the end of the year. Before then, the French plan to sound out other European nations; preliminary discussions with Germany have already taken place.

Judy Redfearn

UK research councils

Getting off lightly

Next year's allocation of the "science vote" of the UK Department of Education and Science is being accepted thankfully by the UK research councils. The thinking may be that if Prime Minister Margaret Thatcher's government can make £200 million cuts in the Conservatives' defence budget, the councils are lucky not to suffer even more.

The Science Research Council, for example, gets £174 million for 1981–82 (at October 1979 prices). Converted to average prices for the current year (say October 1980) this works out at £198 million, compared with current spending for 1980–81 of £204 million — a 3 per cent decrease in real terms. In its annual report for 1979–80, published last November, the council expected "a modest increase" in the next two years; yet a spokesman said this week that the council was "pleased" that the government was treating science and engineering so well.

The Science Research Council's nominal spending on science within the United Kingdom is at present £150.5 million (for 1980–81), and on international subscriptions a nominal £44.5 million. But the latter was calculated at 1979 exchange rates, since when the pound has strengthened sufficiently to reduce the subscriptions bill by some £9 million to

around £36 million. The council has been allowed to use some of this saving to offset its overexpenditure on domestic grants this year (see *Nature* 13 November). Next year, the Science Research Council projects a nominal expenditure of £158.5 million on UK-based science, with £39.5 million on subscriptions (at the same October 1980 domestic prices and exchange rates). Since the pound is unlikely to strengthen further, at least to the degree it strengthened in 1979–80, and the grants to which the council committed itself in 1980 will be continuing, 1981–82 seems set to be a difficult year for SRC.

Among other councils, the Agricultural Research Council has registered a 3 per cent rise from £29.5 million to £30.5 million, and the Natural Environment Research Council remains virtually static at £39.4 million. The Medical Research Council gets £73.2 million, including £13.9 million contract research money recently transferred from the control of the Departments of Health and Social Security. The Social Science Research Council will receive £16.1 million, the British Museum (Natural History) £6.5 million, and the Royal Society £3.3 million. These figures will be adjusted in October this year to account for the inflation rate.

Robert Walgate

Canadian research

Aiming high

Washington

Canada's Liberal Government brought its election promises a step nearer reality last month when it committed itself to increasing the proportion of the country's gross national product spent on research and development from 0.9 per cent to 1.5 per cent by 1985. The commitment is ambitious. Even though the federal government expects a large part of the burden to be carried by the private sector encouraged by tax incentives and other inducements, its own contribution will have to rise from \$1,000 million to \$2,600 million in the next five years — in real terms a growth of 8 per cent a year, allowing for inflation.

Much will also depend on the attitude of the provinces, which through their support of universities contribute about 20 per cent of the nation's effort in research and development. Relationships between the federal and provincial capitals have been cool recently, but officials in Ottawa hope that the need to spur the competitiveness of Canadian industry is one area in which it may be possible to reach agreement.

It will be some time before the broad commitments, announced in Toronto by the Minister of State for Science and Technology, Mr John Roberts, are translated into specific budgets for the various research agencies. And the scientific community is therefore reserving judgement until it sees the colour of the government's money. But for now, the minister's

announcement can be taken as a sign of good faith, since if nothing else it provides a broad framework within which sectoral policies can be worked out.

Spending on research and development has become something of a political football in Canada in recent years, to the surprise of foreign observers who frequently point out that — as the United Kingdom discovered the early 1970s — research spending can be a misleading indicator of industrial strength. Nevertheless, the Canadian public has been convinced that one of the reasons for the country's dependence on foreign investment capital, particularly from the United States, has been its relative lack of an indigenous technological capability. And both the Liberal and Conservative parties have competed with bids to raise research spending to correct the situation.

During last year's election campaign, the Liberals had promised yet again to reach the 1.5 per cent figure, but with no specific target date. Now they have fixed on 1985, and determined that by this time 50 per cent of the nation's research and development spending (equivalent to 0.75 per cent of its gross national product) will come from industry, with a third from the federal government and the rest from the provinces and universities.

Reaching these targets will require a considerable shift in responsibility from the public to the private sector. Industry is being asked to increase its share of the budget from 40 to 50 per cent, while the federal government's contribution would fall in relative terms from 40 to 33 per cent, even though the actual amount of money would increase.

To meet these targets, industry would have to increase its spending by 27 per cent a year — a goal which has already been viewed with a certain amount of scepticism, since the government will have to devise an appropriate set of indirect mechanisms to encourage such growth. Officials in Ottawa are now carrying out a quantitative analysis of the effects of previous policy measures, such as tax breaks on research investment and industrial training grants. One official admitted last week that the studies have so far not been too productive; but there is optimism that some fine tuning can be achieved.

Canada's efforts in this direction bear a close resemblance to the recent domestic policy review of industrial innovation carried out two years ago in the United States under President Carter.

One significant difference, however, is that the Canadian government supports a strongly sectoral approach, identifying areas of high technology — such as nuclear energy, telecommunications or space technology — where it feels that Canadian industry has a particular role to play on international markets.

Although there were structural elements in the policy recommendations put

forward by President Carter eighteen months ago, these were overshadowed by broader proposals aimed at encouraging the general climate for innovation. And with the election of President Reagan, even schemes such as the joint government/industry sponsored Cooperative Automotive Research Project, a pet project of the former president's science adviser, Dr Frank Press, are under threat from free marketeers.

In Canada, the sectoral approach has been strongly supported by groups such as the Science Council, whose chairman, Dr Claude Fortier, wrote in the council's recent annual report that government action is urgently needed along sectoral lines to stimulate innovation and productivity.

This concern is reflected in the government's new proposals. Of the new money which it is proposing to make available for the support of research and development, one half will go to support work in the private sector in clearly defined categories, one third to the government's own mission-oriented laboratories and the rest to universities and other research institutes.

But how much Mr Roberts' announcement indicates a genuine commitment by the Canadian government, already facing a growing budget deficit to increased funding, and how much is merely a political exercise, has become a subject of intense debate.

David Dickson

Environmental cadmium

Europe to ban?

Brussels

Moves to restrict the use of cadmium are gathering momentum in Europe. In Brussels, the European Commission's directive laying down limits for the discharge of cadmium into the aquatic environment is close to being adopted, before it goes to the Council of Ministers, while in Germany, the Home Office Minister has caused a furore in industrial circles by announcing that a new report on cadmium may lead to tough restrictions on its use.

This closely follows news of a Swedish ban due to come into force in July 1983, which has caused much unease among European producers of plastics, stabilizers and pigments, many of which contain cadmium compounds. As a result of research carried out by the Danish National Agency of Environmental Protection, the EEC Council of Environment Ministers has concluded that present environmental levels of cadmium are potentially harmful, the cadmium accumulating in the lungs, bone tissue, and particularly in the kidneys. Cadmium accumulates slowly in man, reaching dangerous levels at around the age of 50. Heavy smokers are thought to be most at risk.

German research has revealed dangerously high cadmium levels in beef, veal,

fish, cereals and potatoes, as well as in some types of mushrooms and mussels. Both Danish and German researchers thought that the cadmium in foodstuffs came mainly from sewerage works through the use of the end product as fertilizer.

The European Commission has previously made strenuous efforts to dissuade the Swedes from making a sweeping ban on cadmium's industrial uses. The Swedish government is phasing out "unnecessary" uses of cadmium, but there are not as yet suitable substitutes for cadmium in its many industrial applications.

According to German Home Minister Gerhard Baum, industry will either have to restrict its use or be faced with a legally enforced ban. The Commission's proposals, if they are finally adopted, will become national law in any case, but as they stand they only serve to protect the aquatic environment, falling short of the restrictions likely in Sweden and Germany.

Two other draft directives on discharge of chemicals into the aquatic environment are already with the council, one on mercury and the other on the "drins" (dieldrin, aldrin and endrin). Other directives are to follow but so far none have been adopted, because member states have failed to agree on limiting values and suitable environmental quality objectives.

The new directive on cadmium will add to the confusion, particularly as it is in two stages, to come into effect in 1983 and 1986, in recognition that the technology for removing cadmium from effluents has still to be developed. One major problem yet to be solved is how to remove cadmium from the wastes created during the manufacture of phosphoric acids from phosphate ore. So until a viable technique is developed, the restrictions will not apply to this industry.

Jasper Becker

Soviet psychology

IQ rehabilitated?

An article in the November/December issue of the Soviet Academy of Sciences publication *Psychological Journal* could pave the way for a controversial about-face by Soviet authorities on the issue of intelligence testing.

Stalin made IQ tests illegal in the Soviet Union in 1936. Before that, over-enthusiastic and under-qualified Russian educational psychologists, working with Western tests not standardized on the local population, had designated massive numbers of rural schoolchildren educationally subnormal — to the dismay and outrage of their parents and teachers.

The article in *Psychological Journal*, under the title "The evolution of cognitive processes and abilities", is likely to be especially influential because its author is Friedhard Klix, president of the International Union of Psychological Sciences and director of the psychology section of the Humboldt University, East Berlin. The

text formed the presidential address to the opening of the Eastern bloc's 22nd International Psychology Conference in Leipzig in July 1980.

The article stresses the need to treat human intelligence as a subject for study in exactly the same way as other biological phenomena. This is in itself a heresy, since Marxist-Leninist theoreticians have always stressed the need to avoid what they called "reductionism", particularly in relation to aspects of behaviour such as intelligence.

Klix places human intelligence firmly in a biological context and subject therefore to the laws of evolutionary genetics; thereby he accepts an indisputable element of heritability. He refers scathingly to the choice (carefully backdated in his example) that has always faced man in attempting to predict and control his own environment — that is, between adherence to a "cult" and the (scientific) alternative of methodical observation. The unspoken inference is that scientists must be allowed to work unhampered by the dogma of political theory.

Klix later refers (under cover of a paragraph concerned with Sumerian mathematical innovation) to recent experiments in his own university department aimed at pinpointing differences in performance between mathematically gifted children and "normals". The Soviet "ukaz" against objective testing, and in particular the prevention of the development of quantitative norms against which children with learning difficulties or exceptional gifts can be measured, is well known to Klix's audience.

Western observers feel that the demands of the Soviet-styled "scientific and technological revolution" may show that this prohibition works against rather than in favour of the very children whom it was intended to protect — selection for special education in one of the schools for gifted children at present depends on performance in one of the so-called "Olympiads". These events are held all over the country, but regularly return an embarrassing over-representation of urban males. The use of tests capable of revealing intelligence potential, uncrystallized by access to sophisticated teaching methods or the enriched cultural opportunities of the city, might restore balance to the selection process.

Klix ends by pointing out that the task before psychology is to achieve for cognitive processes what biochemists have managed in their microanalysis of other biological phenomena. Soviet psychological journals-watchers will be agog to see whether the debate on the "microanalysis" of human intelligence along the lines suggested by Klix is continued within the profession. At least the East Germans — who have been quietly using their own intelligence tests for selection for many years — have provided the Russians with an acceptable (that is, foreign) example on which to base the debate.

Elizabeth Roberts

Orlov hunger strike

Dr Yurii Orlov, the Russian physicist, who is now serving a 7-year prison-camp sentence for his activities as chairman of the Moscow "Helsinki Monitoring Group", has gone on a hunger strike to coincide with the reconvening of the Madrid review conference last week. According to his wife, Irina, Dr Orlov made a similar protest last November, when the conference opened, although, during that month, he served two fifteen-day terms in the punishment cell after an alleged argument with a camp officer.



Yurii Orlov — now a fast

The Madrid Conference on Security and Cooperation in Europe opened last week with the prospect of two months of acrimonious debate (see *Nature* 29 January, p.343).

Dr Orlov's protest, said Irina, is an appeal to all governments represented at Madrid to grant amnesty to political prisoners and to reduce secrecy in the flow in all fields of information — social, economic and military.

So far, however, the Soviet Union seems unwilling to relax its restrictions on information flow. During the Madrid recess, two scientists, Aleksandr Lavut, a Moscow geologist, and Mark Niklus, an Estonian biologist, received sentences of 3 and 15 years respectively for publicizing abroad what they see as breaches of the Helsinki Accords on human rights. "Secrecy", too, is a frequent Soviet excuse for not allowing would-be emigrants to leave the country. The latest to be denied an exit visa on the grounds that he had had access to classified information, is Dr David Goldfarb, who until he resigned his post last year when filing his emigration application had been head of the Laboratory of Molecular Genetics of Bacteria and Bacteriophages of the Soviet Academy of Sciences.

CORRESPONDENCE

Laser war games

SIR — Zoological and anthropological evidence suggests that old conflict systems never die — they are ritualized. Antagonistic relationships are maintained in principle, but rendered progressively less dangerous as destructive behaviour gives way to display.

If increasing proportions of United States and Soviet military budgets are to be devoted to "laser weapons" (*Nature* 15 January, pages 109–111), this trend should be welcomed as a real sign of international relaxation. Strikingly coloured laser beams of spectacular power can be directed harmlessly into space at regular intervals, with none of the unfortunate ambiguity of intention and inelegance of effect which attend the launching of nuclear missiles. In due course, as Capitalist and Communist take their honoured places in history with Royalist and Roundhead, the "Firing of the Lasers" will no doubt become a public spectacle in the same class as the "Changing of the Guard".

EDWARD WHEELER

Newtown, Australia

Exhibition policy

SIR — The debate launched by Dr Halstead on the present orientation of the Natural History Museum at South Kensington, having centred on philosophical issues, leaves aside what I consider the main point. That is that because space has been made for new exhibits, the quality of which I will not discuss, many of the established collections are no longer on display. This was a shock to me when I revisited this institution last autumn for the first time in many years. As a kid in the 'fifties my parents took me to London every year and the Natural History Museum was my favourite place to visit. Those visits were certainly important in shaping my vocation for systematic biology. I especially enjoyed those Victorian cabinets full of shells and insects that present managers have eliminated. I believe that for a child it is more exciting to open drawers full of bugs than to push buttons to see clades lighting up. I believe a natural history museum should be a display of as representative as possible a sample of the taxonomic diversity of the living world. Didactic exhibits on the causes of this diversity (evolution) or the techniques used by systematists should complement the collections and if there is no space available for them, such exhibits could just as well go into the Science Museum.

There is certainly less work involved in preparing displays of stuffed or pickled animals than flashy exhibits on modern biology and maybe the increase (Parkinsonian?) in staff specialized in exhibit preparation, as testified by position openings advertised in *Nature*, is more relevant to the disappearance of collections from the public galleries of the British Museum than are ideologies impregnating the staff.

V. DEMOULIN

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Corrigendum: In the letter "Energetic consensus" from F.H. Malpress, page 335 in *Nature* of 22 January, the units in the third equation should be kcal mol⁻¹, and not kcal.

SIR — How charming that theological and political issues have come back into zoology¹. Halstead² says he is not worried if Marxism creeps into the Natural History Museum, though he does object to "unsubstantiated assertion" while at the same time he objects to different views presented by various sections of the museum — does this not show a due eclecticism on the museum's part?

Halstead's latest contribution² is a direct criticism of the museum's Public Services Department. May I mention my own experience with this department, and its head, Dr Roger Miles? Having been asked some time ago to advise on an exhibition concerned with human perception, I later wrote a fairly detailed criticism of the results, in an editorial of the journal *Perception*³. The response was not the usual rebuttal, but quite the reverse — Dr Miles immediately wrote inviting me to suggest plans for a complete redesign of this exhibition, and to attend the planning meetings and essentially to write the commentaries. This we have done. Whatever the result when it appears, this is evidence that the museum takes criticism seriously and acts with unusual generosity in seeking the help and advice of people outside its staff. Having also just given the museum's Christmas lectures, and so interacted again with the Public Services Department, I must say that in my opinion they have wholly commendable enthusiasm, and scholarship, as well as willingness to accept and act on well meant criticism.

Possibly scientists and writers who have not had experience designing exhibitions do not realize the difficulties. In a book, one can give graded qualifications, and cite rival views; and books are written for particular readerships whose background knowledge and interests may be assumed. But large public exhibitions must cater for children and expert authorities. They should be authoritative and balanced according to present knowledge, while also stimulating naive questions and deep questions for future research. To achieve this with an absolute minimum of text is possible only within limits, which we would all surely like to extend. The Natural History Museum should not merely be preserved as a museum of itself.

R. L. GREGORY

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The Medical School, Bristol, UK

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SIR — May I, as a voluntary worker in the Education Department of the Natural History Museum, add a word to the current discussion in your pages.

I understand as little of cladistics as some of your correspondents seem to, but I wish to put in an ardent plea against the steady destruction of the museum by the Public Services Department. The present policy of tearing up by the roots so many of the excellent, clear, logical and didactically ideal displays and replacing them with gimmicky exhibitions that certainly amuse, but do not instruct, is painful to observe. All is now flashing lights, obscure working-models (all too frequently out of order from over-use by the under-tens), complicated texts that appear on screens only to disappear when you take your finger off the

button before having read or digested them and strange puzzles that nobody has yet understood. These new, trendy, expensive displays, in which, as D. T. Donovan so rightly points out (*Nature* 1/8 January, p.105), original specimens hardly figure or are replaced by colour-photographs or plastic models, reach their tasteless peak in the disco/fun-fair atmosphere of the Human Biology Hall and provide mainly pseudo-information from which little is learnt and even less retained.

I must except from this criticism the excellent new ecology exhibit. It is a pity that it has been relegated to a long narrow hall and disposed in zig-zag fashion so that visitors are constantly colliding as they slalom their way from side to side, missing many of the showcases in the process.

The loss of the remarkable comparative anatomy section as a teaching-aid on adaptation is a tragedy . . . or is its disappearance really a confirmation of more sinister motives?

As for the scattering of the dinosaurs, a logical teaching tour has become a magical mystery tour, with specimens appearing and vanishing from week to week. Poor *Tyrannosaurus rex* remains in splendid isolation in the old hall; if you can find him. Our splendid *Diplodocus* is completely dwarfed by the enormous proportions of the great central hall, yet he cannot even stretch out his tail as in the old gallery, and his head, inserted with difficulty under the bridge, all but protrudes out of the front entrance, where it can only be studied by obstructing and annoying the incoming visitors. This break-up of the fossil collections, for which the right wing of the museum was specifically designed, is a major disaster and if they really mean to dismantle the fossil mammal gallery, I for one will give up and go, much as I love the work. (Name and address withheld — ED. *Nature*)

Soviet rumbles

SIR — Ms Vera Rich has given an inaccurate account of the status of plate tectonics in the Soviet Union (see *Nature* 14 August 1980, p.652). Professor V. V. Belousov (who is a Corresponding Member of the Academy, not a full Academician, and my close personal friend) has no control over what his fellow members of the Academy write or state about plate tectonics. Academician Peyve, also a close personal friend and director of the Institute of Geology of the Academy of Sciences, has published pro-plate tectonics articles with more than 30 of his colleagues since 1966. There are no restrictions whatsoever within the Academy against publishing papers on plate tectonics, and more than 3,000 pro-plate tectonics articles a year are published in the Soviet Union by members, not only of the Academy of Sciences and its branches, but also of regional institutes and academies.

I am an American citizen, not a Soviet citizen, and am fairly well known for my views against plate tectonics. However, I wish to see the scientists of the Soviet Union given their due. Some of the finest pro-plate tectonics literature in the world is being published in the Soviet Union.

A. A. MEYERHOFF

Tulsa, Oklahoma

NEWS AND VIEWS

One gene's intron is another gene's exon

from Piet Borst and Leslie A. Grivell

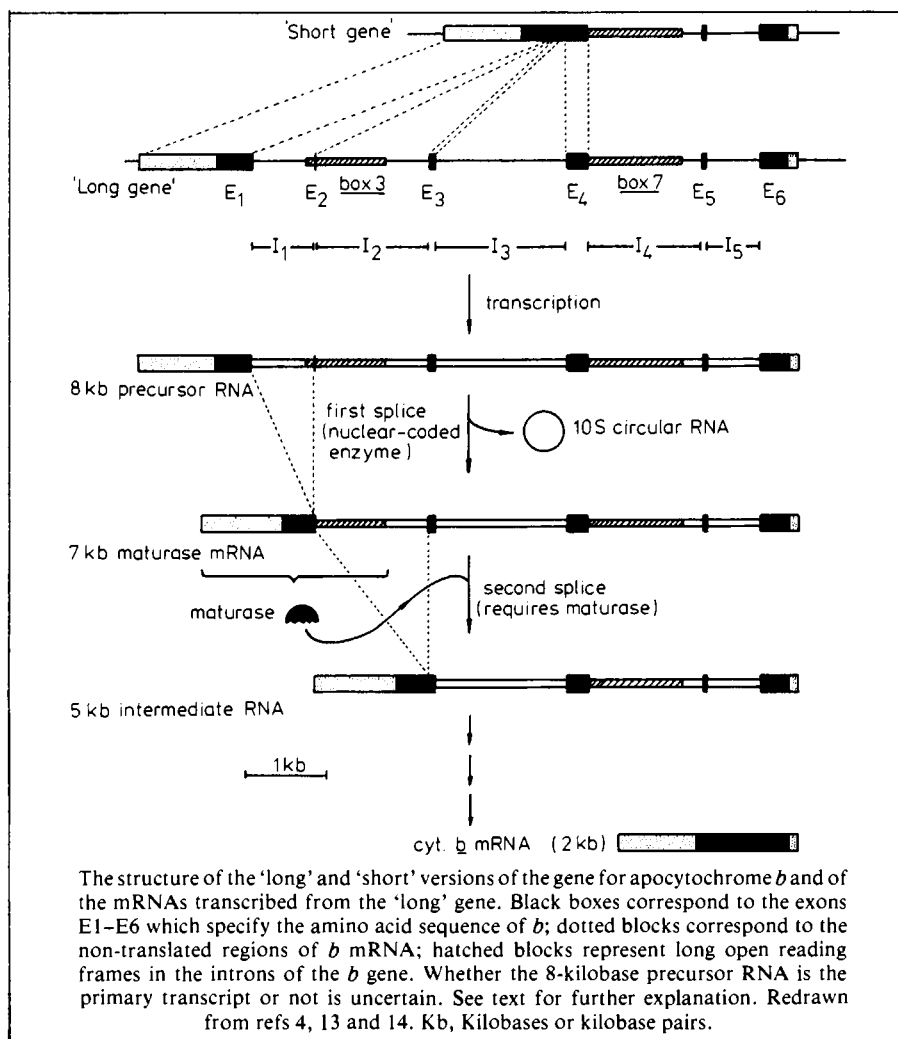
IN 1977 Slonimski and co-workers¹ predicted from an impressive genetic analysis that the gene for apocytochrome *b* in yeast mitochondria would be split and that some of the introns would encode separate functions required for the synthesis of *b* (see review in ref. 2). The split nature of the gene was rapidly confirmed by electron microscopy of DNA-RNA hybrids between this gene and its major transcript, an 18S RNA with mRNA activity for *b* (refs 3,4). The nature of the intron-encoded functions remained elusive, however. Initially, Slonimski pushed the idea that the introns code for 'guide' RNAs, that is, RNAs complementary to exon-intron boundaries in precursor RNA and required for removal of intron sequences^{2,5}. More recently, however, several groups⁶⁻¹² inferred from indirect evidence that some of the introns in the *b* gene code for proteins required for RNA splicing. Although the splicing activity of these proteins remains to be demonstrated directly, a recent paper from Slonimski's laboratory¹³ goes a long way towards proving that their synthesis is essential for the proper processing of *b* gene transcripts.

Our present knowledge of the *b* gene in yeast mitochondria is depicted in the figure. There are two versions of the gene in laboratory yeast strains^{4,14,15}. The 'long' gene contains six exons (E1-6), five introns (I1-5) and two long open reading frames in introns (hatched) which are continuous with the preceding exon. The 'short' gene lacks the first three introns. An early step in the processing of the primary transcript of the long gene is the removal of I1, giving rise to a small circular RNA (10S, refs 5,11,16). Although the exact site of fusion is now known, the effect of this step is to link exon E1 with the tiny (14-base pair) exon E2 (ref. 13) and the open reading frame fused to it. This allows the synthesis of a fusion protein of molecular weight 42,000, called the *box3* RNA maturase by

Lazowska *et al.*¹³. This protein helps to catalyse the excision of the I2 intron and therefore destroys the mRNA which directed its synthesis. Mutations that interfere with maturase function prevent this destruction and lead to overproduction of defective maturase.

The presence of a long open reading frame in the I2 intron was first inferred from the observation that *box3* mutants produce new proteins that look larger than *b* and react with antibodies against *b* (refs 6,7). Since the splicing of precursor RNAs is blocked in these mutants beyond the

excision of the 10S RNA circles (see figure), these proteins must arise by readthrough of ribosomes from E1 into a larger open reading frame in I2. This has now been confirmed directly by Lazowska *et al.*¹³ through sequence analysis of the intron. They go on to show that splicing deficiency in three *box3* mutants is in each case correlated with sequence alterations in the I2 reading frame, both missense and nonsense mutations being effective in preventing splicing. Some of these mutants can be suppressed by paromomycin¹⁷, presumably because this drug induces



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sloppy reading of mRNAs by mitochondrial ribosomes. Since maturase is thought to act catalytically in promoting splicing, a minute amount of misreading should be sufficient to overcome the *box3* mutation.

These results prove that translation of the open reading frame in I2 is required for the proper splicing of precursor RNAs transcribed from the long *b* gene. Two further observations make it clear that maturase is a chimaeric protein, containing the first 143 amino acid residues of cytochrome *b* fused to the I2 reading frame. First, the defective maturase overproduced in *box3* mutants reacts with antibodies against *b* (refs 6,7). Second, chain-terminating mutations in the E1 exon abolish synthesis of maturase^{10,13}. The E1 part of the maturase displays high hydrophobicity, typical of integral membrane proteins like *b*, but may not contribute much to its function since missense mutations in E1 that result in synthesis of defective *b* do not interfere with maturase function^{10,13}.

Whether maturase is a splicing enzyme or only a protein that influences the specificity of other splicing enzymes is not known. In wild-type yeast the protein is present in such low concentrations that it cannot be detected by pulse-labelling (ref. 13 and J. Kreike, personal communication). As pointed out by Lazowska *et al.*¹³, it should be possible to find mutants that over-produce wild-type maturase and a full characterization of this protein should not be long in coming. From the DNA sequence it is clear, however, that the *box3* part of the maturase is rather unusual. It is very basic and hydrophobic, and 170 of the 280 amino acid residues are specified by pure AU-codons (Phe, Leu, Ile, Tyr, Asn, Lys). Yeast mtDNA has a mole per cent G + C of 18 and previous work has shown that there must be a strong selective pressure on this genome to maximize A + T content. In genes coding for respiratory chain components there is a strong preference for A or U in the third position¹⁴; the rRNAs are with 23 per cent G + C the most AU-rich known and intergenic regions are often more than 90 per cent A + T (see ref. 18). The mitochondrial genes coding for major proteins in yeast still contain 27–31 per cent G + C (see ref. 14). Apparently the requirements for maturase function are lax enough to allow the use of 60 per cent pure AU-codons, resulting in a gene containing as little as 18 per cent G + C.

A novel feature in the scheme depicted in the lower half of the figure is that maturase controls its own synthesis, a phenomenon which Lazowska *et al.* call 'splicing homeostasis'¹³. It is likely that this phenomenon is not limited to the *box3* maturase system. The I3 intron also contains a long open reading frame¹⁴; mutations in this frame (*box7* mutants) are blocked in a late splicing step and also produce novel proteins^{10,11,13}. The I4

reading frame may therefore provide the C-terminal part of a second maturase. Several open reading frames have also been found in introns in the *oxi3* region of mtDNA which codes for subunit I of cytochrome *c* oxidase⁹. The set of overlapping transcripts originating from this region is even more complex than the transcripts of the *b* gene^{11,20}. This region may therefore give rise to several additional maturases. Slonimski²¹ has even suggested that splicing homeostasis also occurs outside mitochondria, but this seems doubtful. His proposal requires the existence within nuclei of a protein-synthesizing system capable of reading the opal stop codon UGA as Trp, as mitochondria do (see ref. 22). At present the existence of such a system is hypothetical.

Another remarkable feature of the intricate *box3* maturase system is that it is dispensable. The information in introns I1–3 is present only in 'long' *b* genes and nowhere else in yeast mtDNA (see ref. 3). Hence, strains with short *b* genes make *b* without the intervention of the *box3* maturase. The I1–3 introns belong to a class of 'optional' introns²³ discovered by Sanders *et al.*¹⁵ in yeast mtDNA. Optional introns are also found in the large rRNA gene and in the gene for subunit I of cytochrome *c* oxidase (see ref. 3). Of course, whether an intron is classified as optional depends on finding a yeast strain that lacks it. Since only a limited number of strains have been looked at, all mitochondrial introns may eventually turn out to be optional.

Why should a simple genome like yeast mtDNA with its 70 kilobase pairs have (optional) introns in three genes and none in all the others? It is clear that many of the explanations advanced for the presence of introns in nuclear genes do not apply here (see ref. 23). It seems possible, however, that the introduction of (additional) splicing steps in the processing of precursor RNAs transcribed from key mitochondrial genes could allow a more complete control over mitochondrial gene expression at the RNA processing level. This might be of use in yeast, which can selectively suppress the synthesis of some mitochondrial components, like cytochrome *c* oxidase or *b*, in anaerobiosis or high glucose. This

proposal does not explain the existence of an intron in the rRNA gene, however. Another possibility is that the increased length of intron-containing genes allows more effective recombination within these genes. This could be of advantage in rapidly creating new alleles resistant to yeast inhibitors found in nature. Some of these inhibitors affect *b* (antimycin, mucidin), others the large subunit of the mitochondrial ribosome (chloramphenicol, erythromycin).

Where do these optional introns come from? In their present form they do not have any of the characteristics of insertion elements; each intron is unique and is only found at a single position in mtDNA. It is possible, however, that optional introns have once arisen from insertion elements and that these elements have lost their mobility in the course of evolution. Bacterial transposons usually contain the information for the synthesis of a transposase, which catalyses the replicative transposition of the transposon (see ref. 24). Transposition involves cleavage exactly at the ends of the transposon, analogous to the precise cuts required for the excision of intron sequences from precursor RNAs. The maturases might therefore have evolved from transposases. A transposon which encodes a maturase which can help to excise the RNA transcript of the transposon from larger precursor RNAs could function as a mobile intron. Such mobile introns would allow the effective introduction of new introns into existing genes. It has been suggested²⁵ that introns in mitochondrial genes reflect the presence of introns in the ancestral primitive endosymbiotic bacterium from which mitochondria are thought to have evolved. It is also possible, however, that (some) introns were a later acquisition of the mitochondrial genome. This could explain why some introns in the *b* gene have strong homology with introns in the gene for subunit I of oxidase, even though this subunit and *b* have no clear homology themselves^{14,19}. Such a late acquisition is also compatible with the fact that the introns in the genes for the large rRNAs of yeast mitochondria and *Chlamydomonas* chloroplasts are in different positions in the gene (see ref. 26). □

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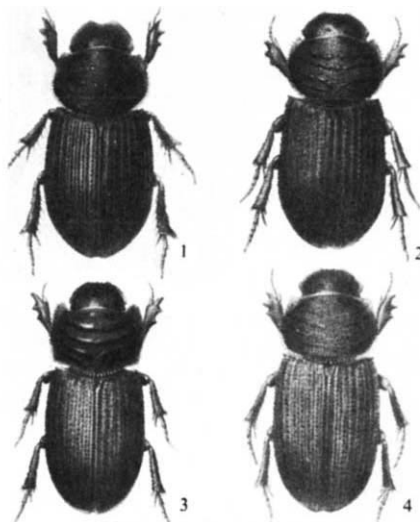
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Beetles in ballast

from R.A. Crowson

IN former times, ships commonly sailed from European ports to North America heavily 'in ballast', filling up with cargo for their return trips. Ballast was generally picked up in the form of sand or shingle from conveniently accessible points on the shore, and discharged in the vicinity of American ports, often in or around the St Lawrence estuary. In the process numerous European terrestrial invertebrates were introduced into a new continent, in which many of them became established. Beetles, probably on account of their exceptional adult longevity and endurance are very well represented among these involuntary colonists, and were particularly discussed by C.H. Lindroth in his book *Faunal Connections between Europe and North America* (1957). One species he did not consider is

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1 *Rhyssalus scaber* Haldeman;
2 *Rhyssalus neglectus* Brown;
3 *Rhyssalus germanus* (L.);
4 *Rhyssalus sonatus* LeConte.

brought to light in an excellent recent revision* of the American species of *Rhyssalus* and *Trichorhyssalus* (Scarabaeidae). The European *Rhyssalus germanus*, apparently first found by the St Lawrence estuary in 1929, is now recorded at several points around the Great Lakes and seems to be spreading. The same species seems to have become at least temporarily established in several other remote parts of the world, no doubt in the same way. As can be seen from the figure, *R. germanus* is not particularly similar to any of the native American species, suggesting that separation of the European and American faunas in this group is fairly old.

*(Gordon and Cartwright *The Western Hemisphere Species of Rhyssalus and Trichorhyssalus*, Smithsonian Contributions to Zoology 317, Smithsonian Instn Press; 1980).

A bright nearby supernova

from Roger A. Chevalier

SUPERNOVA 1979c was discovered on 19 April 1979 in the galaxy M100 by Johnson (*IAU Circ.* No.3348; 1979). The supernova explosion occurred in a spiral arm of the galaxy implying that it was of Type II — related to the explosions of massive stars. The apparent visual magnitude of the supernova near maximum light was 12, making it the brightest Type II supernova since the supernova 1970g. Observations were made at a variety of wavelengths and have revealed new properties of the Type II supernova emission and enabled a new distance estimate to be made for the Virgo cluster of galaxies.

An initial series of coordinated observations at optical, ultraviolet, X-ray and radio wavelengths was undertaken by an international group of collaborators in the first six weeks after the discovery (Panagia *et al. Mon. Not. R. astr. Soc.* **192**, 861; 1980). The observational facilities included the 5-metre telescope on Mt Palomar, the IUE (International Ultraviolet Explorer) satellite and the VLA (Very Large Array) radio interferometer. The optical properties showed the usual characteristics of a Type II supernova: most of the light was in continuum radiation that could be approximately fitted by a blackbody spectrum with a temperature close to 10,000K. Superposed on the continuum were broad lines of hydrogen and other elements in low stages of ionization. The lines developed P Cygni profiles of

emission with blueshifted absorption. If the lines are formed close to the supernova photosphere then the velocity of gas at the photosphere can be deduced from the blue-shift of the absorption minimum to be about 8,000 km s⁻¹.

The IUE observations provided the first UV observations of a Type II supernova. The light was dominated by the continuum radiation, although there was excess emission over the blackbody spectrum at low wavelengths. This may be emission from gas heated in the fast shock wave moving into the material surrounding the exploding star (Branch *et al. Astrophys. J.* in the press). Line features were also observed. Of particular interest were symmetric lines of highly ionized atoms (such as N v, Si iv and C iv) with maximum line widths corresponding to velocities of 4,000 km s⁻¹. The lack of occulting effects in the line profiles implies that the emission was at least two photospheric radii from the centre of the star. Panagia *et al.* suggested that the emission was from a circumstellar shell around the presupernova star which had been accelerated by the supernova radiation.

In the months after maximum, the visual light declined with a time scale of about 20 days. Spectra taken in November 1979 and thereafter showed that the light was dominated by a broad H α line (Branch *et al.*; Kirshner and Chevalier, in preparation). An energy source is required to ionize the gas to produce the H α line and two possibilities are γ rays from the radioactive decay of isotopes synthesized in the

supernova explosion and high-energy emission from a central pulsar. At later times, the IR emission dominated the optical emission. In January 1980, Merrill (*IAU Circ.* No.3444; 1980) found an IR flux about 60 times the flux in the H α line. He suggested that dust formation occurred in the supernova material, but the mechanism that maintains the ionization in the expanding envelope probably also maintains the gas temperature above the condensation temperature for dust formation. Another possible source of the IR radiation is emission by dust in a circumstellar shell that is heated by the supernova radiation (Bode and Evans *Mon. Not. R. astr. Soc.* **193**, 21; 1980). This model requires an absorption optical depth through the circumstellar shell that is close to the upper limit on the absorption set by observations of the supernova near maximum light, but the properties of the shell are in accord with the known properties of mass loss from red supergiant stars, which are thought to be the progenitors of Type II supernovae.

Radio observations with the VLA in April 1979 did not yield a detection, but gave an upper limit of 0.3 mJy (1 mJy = 10⁻²⁶ erg cm⁻² s⁻¹ Hz⁻¹). However, the observations were repeated in April 1980 at a wavelength of 6 cm and the supernova was clearly detected at a level

5 mJy (Weiler and Sramek *IAU Circ.* No.3485; 1980). The minimum brightness temperature of the emitting region was several times 10⁹K if the size of the region was comparable with the radius of the supernova envelope. This indicates that the

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emission was nonthermal. It is likely that Type II supernovae do leave pulsar remnants, which are strong nonthermal emitters. However, the observations of the H α line at the same time as the radio observations indicate that there was sufficient ionized gas to give a large optical depth to free-free absorption. The emission was more likely to be associated with the outer parts of the supernova envelope, but, assuming it was incoherent synchrotron emission, the source of the relativistic electrons and of the magnetic field is not clear.

Besides giving information on the exploding star, observations of the supernova photosphere lead to another important parameter: the distance to the supernova. The technique is to estimate the angular diameter of the supernova photo-

sphere at more than one time from the effective temperature and the total flux from the supernova. The rate of change of the angular diameter, when compared with the actual velocity deduced from the photospheric absorption lines, yields the distance to the supernova. Applying this method Branch *et al.* obtain a distance of 23 ± 5 Mpc. The distance to M100 is especially important because it appears to be a member of the Virgo Cluster, one of the nearest large clusters of galaxies. Distance estimates using more conventional techniques, which depend on nearby distance calibrators, yield distances in the range 12 to 22 Mpc. Distance determinations to supernovae, which are independent of nearby calibrators, should play a fundamental role in determining the distance scale in the universe. □

Negative cooperativity at the insulin receptor

from Alexander Levitzki

IN the past five years P. De Meyts and his colleagues have accumulated an impressive amount of experimental evidence suggesting that negative cooperativity is seen in the binding of insulin to its receptor, that is, as the proportion of cell-surface receptors occupied by insulin increases there is a progressive decrease in the affinity of individual receptors for insulin. In the past two years, however, several reports have questioned this concept and, because the behaviour of insulin may be representative of other peptides and proteins binding to cell-surface receptors, it is of some interest to assess its current status.

De Meyts *et al.* have obtained evidence in favour of negative cooperativity both from an equilibrium binding approach and from measuring the rate of dissociation of 125 I-insulin as a function of increasing receptor occupancy. Furthermore, in a study of twenty-four different insulin analogues they showed that while some insulins bind with negative cooperativity, others do not reveal negative cooperativity in either binding or in their kinetics of dissociation in the same experimental system¹. The recent objections to invoking negative cooperativity as the explanation of the data accumulated by De Meyts *et al.* have been of three main kinds.

First, several observations seem to suggest that the enhanced 125 I-insulin dissociation at increasing occupancy is not an unambiguous proof of the negatively

cooperative model. For example, 125 I-TSH (thyroid-stimulating hormone) binds in a non-cooperative manner to cultured thyroid cells, giving linear Scatchard plots, but its rate of dissociation from the receptor is enhanced by increasing concentrations of the unlabelled hormone². Similarly, the enhancement at 10 °C of the dissociation of 3 H-dihydroalprenolol from frog β -receptors by native alprenolol occurs at a range of ligand concentration that yields non-cooperative binding (Hill coefficient of 1.0)³. In addition, recent studies by Pollet *et al.*⁴ reveal no negative cooperativity in the same frog erythrocyte β -receptor system studied by Limbrid *et al.* An enhanced rate of dissociation has also been observed for biologically inert substances. For example, insulin enhances the rate of dissociation of 125 I-insulin from talc⁵ and similarly, the rate of dissociation of 125 I-abrin from Sephadex particles is enhanced by unlabelled lectin or lactose⁶. In addition, difficulty arises from the claim of Pollet *et al.*⁷ that no enhancement of 125 I-insulin dissociation is obtained at increasing receptor occupancy in the same IM-9 lymphocytes as those studied by De Meyts *et al.* The latter findings are in direct conflict with those reported by De Meyts and his colleagues⁸ which were recently confirmed, on the same cell line, by Sonne and Gliemann⁹. The main conclusion from these different studies is that one must recognize that the existence of an enhanced dissociation of a ligand from its receptor in the presence of excess ligand, in cases where negative cooperativity clearly does not occur, eliminates this method as a

definitive tool. It has been recently suggested how it might be possible to obtain a definitive interpretation by means of a new type of equilibrium measurement¹⁰.

A second objection is raised by a recent study which claims that the existence of high- and low-affinity sites represents heterogeneity and not negative cooperativity¹¹. The authors demonstrate that the binding isotherm of 125 I-insulin to human red cells is fitted well by a model which assumes two classes of sites: each cell reveals one high-affinity site ($K_d \sim 71$ pM). The authors further show that the high-affinity site can be completely blocked by the binding of concanavalin A, leaving the low-affinity sites intact. This finding is not compatible with the negative cooperativity model of De Meyts *et al.* One must remember, however, that a sample of human erythrocytes is always heterogeneous, certainly in their age. It would not be surprising if one were to find that the high-affinity sites and the low-affinity sites do not reside on the same erythrocyte, in which case the entire argument of Herzberg *et al.* is invalid. It is also difficult to use an experimental system that exhibits only one high-affinity site per cell and in which it is not clear whether the insulin receptors are functional.

The final complication was pointed out recently by Donner¹². 125 I-insulin is degraded rapidly by intact hepatocytes into 125 I-insulin fragments. These fragments possess a spectrum of affinities that is manifested in curvilinear Scatchard plot for 125 I-insulin binding to the intact hepatocytes. When the authors correct for the true intact 125 I-insulin, the binding pattern is converted into a non-cooperative one, represented by a linear Scatchard plot. Thus, when one does not correct for 125 I-insulin, one observes about 30,000 high-affinity sites per cell ($K_d \sim 1.0$ nM) and about 600,000 sites per cell ($K_d \sim 60$ nM). When such a correction is made, only 36,000 sites per cell ($K_d \sim 0.4$ nM) are observed. These results also fit with the common observation that the biochemical response of these cells to insulin is of a non-cooperative Michaelian type. The authors, in fact, confirm earlier reports¹³ in which it was reported that 125 I-insulin is degraded to fragments by intact hepatocytes.

Although Donner's findings are relevant to intact hepatocyte cells which are obviously loaded with degradative

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enzymes, his conclusion cannot be extrapolated to the purified membrane systems studied by De Meyts and his colleagues, and most probably does not apply to other cell systems. Indeed, only recently Sonne and Gliemann⁹ have shown that ¹²⁵I-insulin is not degraded at all by the IM-9 lymphocytes, even at 37 °C. This cell line exhibits strong negative cooperativity towards ¹²⁵I-insulin, as judged both by binding studies and by accelerated dissociation of ¹²⁵I-insulin^{1,9} at all temperatures including physiological ones.

To summarize, the most important point raised by the critics of the negative cooperativity model for insulin receptors is the demonstration that the accelerated rate of dissociation of a ligand from its receptor at increasing occupancy is not a definitive proof for negative cooperativity. Thus, in order to demonstrate negative cooperativity (or its absence) one must resort to other methods as well. For insulin itself, it seems that the experimental evidence for negative cooperativity in quite a number of systems is still very convincing. □

The Trigger experiment: simulating auroral processes

from Daniel W. Swift and Hans C. Stenbaek-Nielsen

TO INVESTIGATE the possibility of triggering auroral processes, a rocket experiment, appropriately code named 'Trigger', was designed and carried out by a group of Swedish and United States auroral researchers. In Trigger the electrical properties of the ionosphere were artificially perturbed by an explosive release of caesium metal and the effects were observed by a wide range of both rocket-borne and ground-based instruments. The experiment took place in February 1977 with launch from Esrange near Kiruna, Sweden, and indeed, the results have recently appeared in the *Journal of Geophysical Research* (10 October, 1980).

Traditionally, the ionosphere has been considered an entirely passive medium and the aurora to be little more than the ionospheric imprint of processes taking place out in the magnetosphere. But the ionosphere is electrically coupled to the magnetosphere with large currents flowing between the two regions and recent data indicate that these two currents are important for auroral processes. Thus it would seem reasonable to expect that events taking place in the ionosphere may affect magnetospheric processes. Evidence for a less passive ionosphere has also been found in observations on some chemical releases, although these experiments were never intended to produce such effects; an example is pulsating auroras apparently stimulated by a barium release (Deehr and Romick *Nature* **267**, 135; 1977).

While we have long known that the optical emissions forming the aurora are for the most part due to energetic electrons precipitating into the upper atmosphere, it is only within recent years that we have begun to understand the mechanisms which are responsible for this precipitation. The authors are in the Geophysical Institute at the University of Alaska.

and the role played by the ionosphere.

It appears that two general processes are responsible for the precipitation of auroral electrons. The first process is the result of leakage of energetic electrons from the Earth's radiation belts into the atmosphere. This leakage is greatly enhanced by very low-frequency radio emissions and electrostatic waves which can be generated spontaneously within the radiation belts or stimulated from the outside. Processes of this type were among the earliest suggested and are to a large extent responsible for the diffuse aurora.

The other category of processes is associated with the discrete aurora, for example, the spectacular arcs and bands

often observed in the late evening and along the poleward edge of the auroral oval. The main characteristic is potential differences set up along the magnetic field lines in which electrons are accelerated in much the same way as in the electron gun of a television tube. These potential structures are located in the near-Earth magnetosphere and their existence has only recently been verified experimentally.

Although the Trigger experiment did not actually create an artificial aurora, measurements of energetic electron fluxes in the neighbourhood of the plasma release gave indications of both types of auroral precipitation processes.

The caesium plasma was created by a 12-kg mixture of TNT-AIO-CsNO₃ which was explosively released at an altitude of 164 km. Caesium is a metal that easily ionizes to form a plasma and thus the release resulted in the rapid motion of an electrically conducting plasma cloud across the Earth's magnetic field. The motion of a conductor across the magnetic field lines constitutes an electrical dynamo which generates electrical voltages capable of accelerating electrons. In addition, the Trigger experiment creates a number of waves which may travel out into the magnetosphere and it leaves in its wake an enhanced level of ionization that can also affect plasma processes which occur on magnetic field lines passing through the plasma cloud.

An instrumented payload was sent up with the payload containing the explosive charge, but separated from the charge before the burst, so that when the explosion occurred, it was separated by 1.5 km and on a magnetic field displaced by about 0.5 km from the instrumented payload. A large peak in the electric field

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Photograph taken from the optical observing site at Andoya Rocket Range, northern Norway, of a natural aurora showing auroral arcs (with a little local cloud in front of them), which stretched from the eastern horizon to the western horizon (behind the mountains). (Taken by H.L. Collin of the Geophysical Institute.)

was seen about the time the blast wave should have crossed the magnetic field line intersecting the payload. Nearly coincident with this, electron fluxes of 2-kV were seen moving up and down the field lines, but the electrons did not actually seem to reach the atmosphere. The currents generated by the electric field apparently set up reflecting electric shocks both above and below the burst point, and the electrons were probably trapped between them.

The discrete auroras are believed to be caused by electrons accelerated through similar shocks, or double layers, also set up by currents flowing parallel to magnetic field lines; but although the current-driven shocks play roles in both the natural aurora and the Trigger experiment, their roles are quite different.

The instrument package also observed electron fluxes with energies greater than 40 kiloelectron volts (keV). There was a prompt burst followed a second later by sustained fluxes of electrons. Fluxes greater than 40 keV are believed to be responsible for the patchy and pulsating aurora seen in the early morning hours in the diffuse aurora (see review by A.D. Johnstone *Nature* 119, 274; 1978). The natural precipitation is also accompanied by electromagnetic wave emissions called chorus. The chorus emission is in the audio frequency range, 0.5 to 2 kHz, and when

amplified sounds like the chirping of birds. The precipitation can be enhanced by increasing the ambient ionization because the ambient ionization makes the trapped electrons more unstable to precipitation caused by the electromagnetic emissions.

It is tempting to attribute the delayed precipitation of electrons of energy greater than 40 keV in Trigger to the enhanced ionization density left by the caesium in the wake of the Trigger blast wave. The delayed fluxes were accompanied by wave emissions, but the emissions were electrostatic, rather than electromagnetic, and so would not probably be able to cause precipitation of trapped fluxes. Moreover, the Trigger fluxes were primarily field aligned, which is again inconsistent with a wave-particle diffusion process. The experimenters attribute most of the enhancement to noise contamination of the magnetic field-aligned detector. However, an X-ray detector suspended from a parachute that was deployed by a second rocket did observe 0.13-Hz pulsations in X-ray fluxes some two minutes after the event. This frequency is comparable with pulsation frequencies observed in natural aurora.

The prompt energetic electron fluxes are believed to be real, and they are accompanied by an electromagnetic pulse. However, as the electron fluxes are also primarily field aligned, the acceleration

mechanism would have to be a stochastic process, since the electric potentials generated by the explosion are considerably less than the observed electron energies.

The Trigger experiment initiated a number of interesting plasma processes, some of which are similar to those responsible for the aurora. However, the natural aurora and the events observed in Trigger are not similar enough for the Trigger results to be applied in a direct way towards understanding the aurora; but after further theoretical interpretation it may turn out that the experiment has provided a number of useful clues to understanding processes in the natural aurora.

Certainly, the concept of the Trigger experiment is very appealing. Not only does the technique of artificially perturbing the ionosphere or magnetosphere allow the study of a wide range of general plasma processes, it also makes it possible to separate cause and effect, an often serious problem in the interpretation of auroral observations.

The use of chemical releases in space research will increase; groups from many countries are now proposing that such release experiments be conducted from a 'chemical release module' which will be launched through the US space shuttle programme sometime in the mid-eighties. □

Are membrane proteins introverted?

from N. Michael Green

THE PURPLE MEMBRANE of *Halobacterium halobium* has become a test bed for sophisticated new techniques. These, in turn, have given exciting new information about the structure and function of the major protein of the purple membrane, bacterial rhodopsin. The most recent technique, neutron diffraction, shows that rhodopsin is an "inside-out" protein. Six years ago a combination of novel electron microscopic techniques with X-ray diffraction gave a picture of the structure at 7 Å resolution (Henderson and Unwin *Nature* 257, 28; 1975). Then last year, a correlation of the electron density distribution with the amino acid sequence of appropriate helical segments produced a tentative model structure (Engelman *et al. Proc. natn. Acad. Sci. U.S.A.* 77, 2023; 1980). It is this model which now receives strong support from some elegant neutron diffraction experiments (Engelman and Zaccai *Proc. natn. Acad. Sci. U.S.A.* 77, 5894; 1980). This method, based on the great difference between neutron scattering by protons and deuterons, has also been used to show that there are no detectable aqueous channels in the membrane (Zaccai *J. molec. Biol.* 132, 181;

1979; Rogan and Zaccai *J. molec. Biol.* 145, 281; 1980.).

Purple membranes were prepared in which all of either the valine or the phenylalanine residues had been replaced by their fully deuterated analogues and the neutron diffraction patterns were compared with that from normal membrane. From the latter a neutron density map was constructed which closely resembled the electron density map. Difference Fourier maps relative to the deuterated preparations were computed and showed that valine and phenylalanine residues were concentrated in different regions. This was to be expected, since these residues have rather different distributions both between the seven transmembrane helices and azimuthally within each helix. The peak density differences for both valine and phenylalanine side chains were off the helix axes, as would be expected, but whereas the valine peaks were displaced towards the exterior of the molecule those of phenylalanine were nearer to the interior, in the region where many of the charged groups had been placed in the tentative structure of Engelman *et al.* This differential distribution was consistent with the relative azimuthal distributions of valine and phenylalanine derived from the sequences of the previously assigned helices.

Comparison of the sequence-based azimuthal distributions with those of the charged residues confirms that valines (17 out of 19) are on the opposite sides of helices from phenylalanines (9 out of 11), and that the buried charged groups go with the phenylalanines. Moreover, the previously assigned locations of helices rich in valine or phenylalanine were consistent with the Fourier difference maps derived from neutron scattering, though the authors refrain from any detailed interpretation of this at the moment. The consistency of these new results with the earlier model is impressive and gives confidence in the validity of the arguments used in support of the previous assignments.

A firm picture is beginning to emerge of an 'inside-out' protein molecule with buried polar and charged groups located predominantly between helices and with most of the interface between lipid methylene groups and the protein being occupied by hydrophobic aliphatic chains. The authors suggest that in addition to a possible role in providing sites for proton translocation, the charged and polar groups would be an important stabilizing element in such a predominantly hydrophobic structure, since in their absence interactions between hydrophobic helices immersed in a hydrophobic solvent are very weak.

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African lithosphere

from Paul Mohr

ONE reason for the temporary demise of continental drift as a respectable scientific hypothesis was that the rigidity of planet Earth appeared too great for such drift to be possible. However, by 1948 the seismologist Beno Gutenberg was able to confidently state that a 'Low Velocity Zone' existed within the Earth's upper mantle. From seismic shear-wave velocity measurements the zone is estimated to extend between depths of about 120 and 200km. Its physical properties are consistent with incipient melting of mantle peridotite rock, and thus the term asthenosphere (zone of weakness) is appropriate. The overlying lithosphere, comprising the crust and topmost mantle, contrasts physically with the asthenosphere in being stronger and more rigid. The lithosphere has become identified with the plates of plate-tectonic theory and these plates are believed to move over the asthenosphere.

The global pattern of motions of the lithospheric plates has now been measured (see ref.1) and the manner of plate generation at sea-floor spreading axes, together with the ensuing thickening of the sub-oceanic lithosphere are well known. Much less well known is the thickness of the lithosphere under continental sectors of plates, and the means by which such lithosphere becomes attenuated where a plate begins to split, as, for example, along the African Rift System.

Early insights into African lithospheric thickness came from surface-wave dispersion studies²⁻⁵ and in 1976 one could say, in summary, that under the undeformed interior of Africa, the top of the asthenosphere lies near 120km depth, but near the margins of the rift system it rises to an average depth of about 80km. Thin though the data were, with the addition of seismic profiling studies of the African crust they provided essential constraints for interpreting the more extensive data derived from other geophysical techniques.

Gravity surveys in eastern Africa have firmly established that lithospheric thinning is associated with the rifting of a continent. Sowerbutts⁶ proposed the existence in Kenya of a low-density asthenospheric 'cushion' underlying an unthinned lithosphere, but Girdler *et al.*⁷ explained the same gravity data solely in terms of lithospheric thinning. More recent work has led to a combination of these two ideas, using a starting thickness for the unattenuated lithosphere of 80-100km⁸ or

120km⁹. The asthenospheric 'cushion' beneath the Rift System may have a prism-like cross-section, according both to seismic array¹⁰ and geomagnetic sounding¹¹ studies, with the lithosphere-asthenosphere boundary initially dipping steeply away from the rift valleys.

A particularly bold contribution to African lithospheric studies has come from combining continent-wide gravity data, based on the 1973 US Defense Mapping Agency's Bouguer-anomaly map of Africa, with observed teleseismic delay-times for Soviet underground nuclear explosions recorded at African seismological stations. Fairhead and Reeves¹² resulting map of lithospheric thickness, derived from empirical linear relationships among delay-time, station elevation and Bouguer anomaly, reveals a broad zone of thinning coincident with the Rift System, from Ethiopia through Kenya and Tanzania to Angola. To the northwest, across Zaire and the Sahara, the lithosphere purportedly averages 200km thickness (less for the Tibesti-Jebel Marra line), while in southern Africa, southeast of the Rift System, the thickness lies between 175 and 150km. These values are indeed appreciably higher than indicated from the more direct approach of surface-wave dispersion analysis.

An almost identical picture, but with very different absolute values for lithospheric thickness, is most recently provided from a reinterpretation of the US Defense Agency gravity map following on a convincing demonstration of the accuracy of the map^{13,14}. Detailed analysis shows that lithospheric thickness associated with the rift valleys and their bordering plateaux is less than 50km, and is less than 70km for a substantial part of southern Africa. These values are significantly less than those derived from seismic-wave studies.

How can such divergent conclusions be reached from essentially the same data base? One obvious reason is that seismological constraints are still not adequate to provide a firm model of density-depth gradients, and thus facilitate a unique interpretation of gravity profiles. Brown and Girdler¹⁴ agree with Fairhead⁸ in obtaining a density contrast of 0.05g cm⁻³ at the lithosphere-asthenosphere boundary, but in retaining the fundamental assumption of Girdler *et al.*⁷ that the longer-wavelength Bouguer anomalies of Africa are caused entirely by variations in lithospheric thickness. That is to say, there is no contribution to the gravity-field from lateral changes in density below the lithosphere — there is no asthenospheric 'cushion'.

What is the evidence for an asthenospheric 'cushion', that is, for steeper and more profound asthenospheric boundaries than thus far provided by any of the published gravity interpretations? Seismic-array investigations in Kenya indicate that the sub-Rift System asthenosphere remains laterally distinct from 'normal' asthenosphere down to depths of 250km, far below the base of even unattenuated lithosphere¹⁰. The asthenosphere beneath the Rift System could therefore be hotter, more melted and even less dense than 'normal' asthenosphere under the rest of Africa. Here we approach the crux of the problem.

All agree that volcanism and high heat-flow in Africa are associated with zones of lithospheric thinning. But is this merely the result of passive upwelling of asthenospheric material as the plates begin to separate? This thesis is the rationale for and the explicit conclusion of Brown and Girdler's¹⁴ interpretation of gravity. It is also the conclusion, reached on the entirely different premises of isotope geochemistry of volcanic rocks in Afar, of Barberi *et al.*¹⁵. Alternatively, are there 'hot spots' or deep mantle plumes impinging on the underside of the African lithosphere, involving vertical mass-transfer that could well derive from the mesosphere at depths of 400km or more^{16,17}. This crucial issue is unresolved.

The questions of how thick is the African lithosphere, and the manner of lithospheric thinning as Africa splits, remain to test the best of the next generation of earth-scientists. Further questions arise: How deep and wide is the column of mantle perturbation below the Rift System, and do phase-changes below 400km depth contribute to the gravity field? What is the variation with depth of lateral density gradients within the asthenosphere under eastern Africa? And closer to the surface:

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How does plateau crust, thickening as the rift margins are approached, join with the attenuated crust of the rift floors? Does the rift Moho, evolve in nature and depth in the way proposed by Mueller¹⁸? Finally, is it possible that the detailed gravity signature for Africa expresses sublithospheric mantle convection (as postulated on

other grounds by Thiessen *et al.*¹⁹ with horizontal dimensions of about 1,000 km? A rather similar figure has recently been derived from bathymetry-geoid measurements for the Pacific by McKenzie *et al.*²⁰, hinting that mantle convection beneath oceans and continents is of similar if not identical form. □

Is fibroblast interferon antigenic for man?

from D. A. J. Tyrell

In this issue of *Nature* (p.496) Vallbracht *et al.* report the treatment of a child with interferon β for the malignant disease, neuroblastoma. Interferons are known to act against experimental tumours, possibly by inhibiting cell division and enhancing immune activity, for example by activating natural killer cells. As fibroblast interferon is not usually found in the circulation after intramuscular or subcutaneous injection, possibly because it is bound by tissues, it is given intravenously when a systemic effect is required in clinical studies. In the case reported by Vallbracht *et al.* there was a poor clinical response and while monitoring the pharmacokinetics of the injected interferon it was found that there was no interferon in the blood. An anti-interferon globulin was detected in the circulation, apparently in consequence of

the repeated interferon injections. This did not appear in other patients. What does this mean?

From analysis of cloned human genes it is clear that interferons are families of molecules. Apparently there are distinct genes for eight different forms of interferon α (IFN- α or leukocyte interferon), and IFN- β (fibroblast interferon) may also be the product of more than one gene. All interferons do, however, seem to be 'poor' antigens — in the practical sense that it is difficult to make antisera against them.

Nevertheless, antibodies against interferons have been produced by inoculating them into foreign species, and in a recent example a mouse immunized against human IFN- α — exactly which one we do not know — provided a monoclonal antibody-producing hybridoma. Immunoglobulin from the hybridoma has been used to purify interferon for clinical study (Secher and Burke *Nature* 285, 446; 1980),

and may also be used for radioimmunoassay.

One would not expect to be able to produce antibodies by inoculating an interferon into the same species as the one which produced it. In general, immune responses are not mounted against normal constituents of cells because of a 'self/non-self' recognition mechanism which distinguishes between normal tissue components and novel and presumably extraneous antigens. However, as a transient response to tissue damage and as part of the 'autoimmune' diseases antibodies can be produced against normal tissue constituents. This reaction seems particularly likely when the tissue component, for instance thyroglobulin, does not ordinarily enter the circulation and encounter cells of the immune system.

Did the patient treated by Vallbracht *et al.* have some impairment of the self/non-self mechanism and thus react in this way? Is IFN- β normally found only in or near to producing cells, so it is recognized as foreign when it enters the circulation? Was the IFN- β given subtly different from the patient's own IFN- β , perhaps because it was the product of a different gene or because it was modified or denatured during manufacture? It remains to be seen whether anti-interferon antibodies are anything but an occasional nuisance when trying to treat patients with interferon — no such trouble seems to have arisen during the treatment of substantial numbers of patients with IFN- α . However, the single case report stimulates us to ask questions about the biology of interferon which we might otherwise not have thought of asking, and that must be a good thing. □

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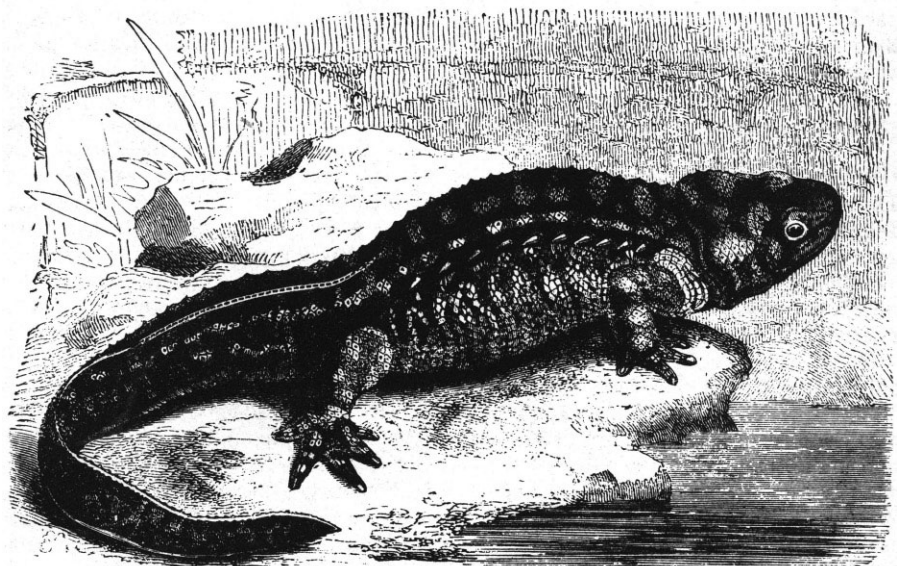


100 years ago

DR. LENZ, the German traveller who lately accomplished the feat of reaching Timbuctoo from the north, has arrived at Bordeaux, and is expected at Berlin soon to give an account of his explorations.

News from Cairo states that to the north of Memphis, near Saggarah, two pyramids have been discovered which were constructed by kings of the sixth dynasty, and the rooms and passages of which are covered with thousands of inscriptions. The discovery is said to be of the greatest scientific importance.

The habits of the *Pleurodeles* seem to be more or less like that of our native Tritons. During the procreative season they remain upright in the water; later they leave it and hide themselves in damp places under stones. Like the Water Newts, they possess a sort of cry; when frightened, as on being suddenly seized,



Pleurodeles waltlii

they emit a low, short, almost squeaking sound, generally repeated several times. This seemed to come not so much from the throat as to be caused by a rapid expulsion of air through the openings of the nose — in fact, to be a sort of snort.

It would almost seem worth one's while to

pay a visit to those Andalusian tanks, and by their semi-limpid sides and under the shelter of their surrounding fig and olive-trees work out the complete history of this interesting little form.

From *Nature* 23, 3 February, 1881.

ARTICLES

Apparent thickness of Saturn's rings

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The determination of the physical thickness of Saturn's rings is crucial for a better understanding of the nature, the dynamics and the evolution of a system of colliding ring particles. Ground-based observations with electronographic cameras and CCD during the transit of the Earth through the ring plane in March 1980, reveal a photometric apparent thickness of 1.4 ± 0.3 km. This value is only an upper limit of the local ring thickness, which is not observable from the Earth or from flybys. The observed brightness includes the contribution of the E ring, of large chunks and condensations and of the warping of the disk. Theory predicts a local thickness of the order of only a few mean particles radii.

WE know of three dissimilar systems of planetary rings. Saturn has bright, broad rings separated by narrow gaps; the rings of Uranus are dark, narrow and widely spaced while the main jovian ring, closer to its central planet, is intermediate in size and has a three-dimensional halo. These rings are the nearest disk-like systems we have, and should offer valuable insights into the dynamics of more exotic and less accessible flat systems such as spiral galaxies, accretion disks and the primordial Solar System.

Following the discoveries of Uranus' and Jupiter's ring in March 1977 and March 1979, respectively^{1,2}, Saturn's system is now being intensively observed. Pioneer 11 became the first spacecraft³ to reach Saturn on 1 September 1979, and the Voyager spacecraft has produced much interesting data, and more discoveries can be expected on its next encounter (27 August 1981). In between these encounters, ground-based observations during the ring plane crossing have revealed new inner satellites^{4,5,7}, new rings^{6,8}, and that the surroundings of Saturn are much more 'crowded' than foreseen.

Observations at very low elevations may supply important new information on the shape and the nature of the rings. In particular, when the rings are seen edge-on as a very faint line, the measurement of the brightness of this line may enable us to estimate the physical thickness of the rings.

Photometric determination of the thickness of Saturn's rings

The main problem is the evaluation of the physical thickness z of Saturn's rings, which lies far below the optical resolution of any instrument ($z \sim 1$ km whereas the resolution, from the Earth, is $\sim 6,000$ km). Nevertheless, photometric measurements of the rings, even though taken close to a much brighter planet, enable z to be determined.

On the plates we have examined, the Sun and the Earth are on opposite sides of the ring plane, so that the side S we can observe is dark in comparison with the edge E (Fig. 1a). The plates we have reduced were taken by Laques *et al.*⁹, with the 1.06-m reflector of the Observatoire du Pic-du-Midi, and by Wlérick *et al.*¹⁰, with the 1.93-m reflector of the Observatoire de Haute-Provence. In both cases, the receiver was an electronographic camera built at the Paris Observatory. Table 1 summarizes the reduced observations and provides details about receptors and conditions of observations. A microdensitometry of these plates provides ϕ , the luminous flux per unit length (1 arcs), of the rings (Fig. 1a). This flux is compared with that of a satellite which is not overexposed on the plate. Depending on the plate, the reference satellite is Tethys, Enceladus, Hyperion or Mimas. Our photometric measurements agree within an accuracy of 0.15 mag with known data¹¹.

By using the simplest model of the rings, that is, a plane parallel homogeneous scattering layer with a rectangular cross-section¹² in which light undergoes multiple isotropic scattering, it is then possible to separate ϕ into two parts: ϕ_E , the flux from the edge E, and ϕ_S , from the dark side S. In the ideal case where the rings are just seen edge-on, we obtain $\phi = \phi_E$. Actually this is not observed, so we had to extrapolate $\phi = \phi_E + \phi_S$ from the observed geometries to its minimum value ϕ_E at the edge-on geometry.

For this extrapolation we then need b_E , the brightness of the edge, or the mag/arc s² m_E of the edge ($b_E = 10^{-0.4m_E}$) because $z(\text{arc s}) = \phi_E/b_E$.

The tables in ref. 13 provide observed brightness m_v and m_B (mag/arc s²) of the illuminated side, in visible and blue light respectively at different points of the rings, with a theoretical estimation of τ (perpendicular optical thickness) at these points. The elevation angles B of the Earth and B' of the Sun are then equal to $B \approx B' \approx 26^\circ$, and the phase angle $\alpha = 0.094^\circ$. A large optical thickness is derived at the brightest part of B ring ($\tau \approx 1$),

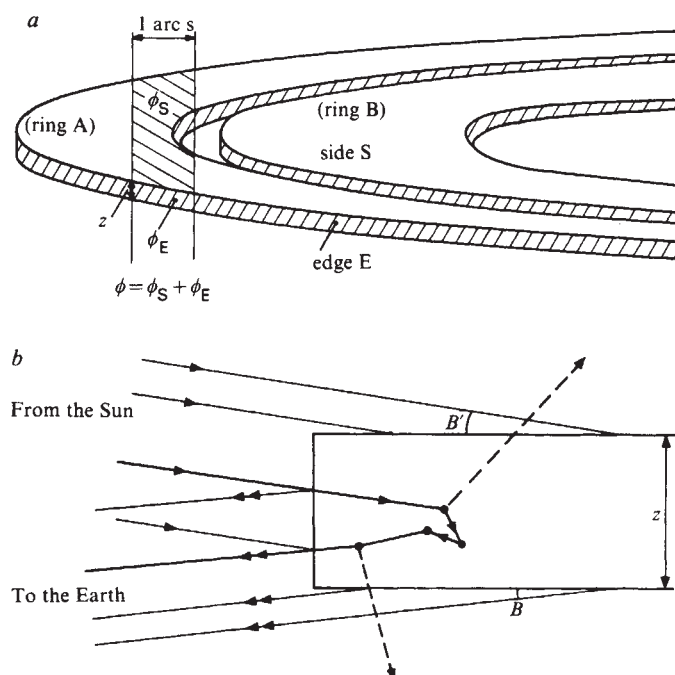


Fig. 1 a, Plane-parallel model of Saturn's rings. The thickness has been magnified compared to radial dimensions. b, The tilt-angle B is much smaller during the 1980 observations so that the rings appear as a line only.

Table 1 Summary of the reduced observations

Identification	Date	μ	μ_0	$B \times B'$ (deg) ²	α (deg)	Colour	Mag/arc s of a ring segment		Adopted reference
							17 arc s	20 arc s	
PbY 136	80 03 12.94	-0.000168	0.00264	-0.00145	0.29	V	15.5 (W)	15.2 (W)	V_0 Tethys = 10.3
PbY 137	80 03 12.95	-0.000178	0.00264	-0.00154	0.29	V	15.6 (E) 15.5 (W)	15.2 (E) 14.7 (W)	V_0 Hyperion = 14.2
PbY 145	80 03 16.01	-0.00220	0.00346	-0.0249	0.34	V	15.7 (W)	14.7 (W)	V_0 Hyperion = 14.2
PbY 152.C	80 03 17.01	-0.00286	0.00372	-0.0349	0.41	V	14.7 (E) 14.9 (W)	14.7 (E) 14.8 (W)	V_0 Hyperion = 14.2
PbY 152.E	80 03 17.01	-0.00286	0.00372	-0.0349	0.41	V	15.6 (E) 14.9 (W)	14.7 (E) 14.4 (W)	V_0 Enceladus = 11.8
PbY 154	80 03 17.05	-0.00288	0.00374	-0.0353	0.41	V		14.7 (W)	V_0 Hyperion = 14.2
SATU 29.11	80 02 29.18	0.00826	-0.000799	-0.0219	1.58	NF	15.3 (E)	15.5 (E) 15.7 (W)	B_0 Mimas = 13.5
SATU 18.01 B	80 03 18.04	-0.00351	0.00400	-0.0460	0.51	NF	14.7 (E)	15.2 (E) 14.8 (W)	B_0 Mimas = 13.5
SATU 18.07 A	80 03 18.10	-0.00358	0.00401	-0.0472	0.52	B	14.8 (E) 14.8 (W)	15.3 (E) 15.0 (W)	B_0 Tethys = 11.0

$\mu = \sin B$ and $\mu_0 = \sin B'$ where B and B' are the elevation of the Earth and the Sun respectively above the ring plane. α is the phase angle. NF, no filter; we used B mag to derive a value of the thickness. (E) and (W) refer to eastern and western ansae of ring. The saturnicentric distances of Sun and Earth were respectively 9.44 UA and 8.45 UA over this period.

and thus the limits of $m_v(\infty)$ and $m_B(\infty)$ when τ is infinite are easily obtained from these data. We found $m_v(\infty) = 6.32$ and $m_B(\infty) = 7.22$. These values have to be corrected from solar distance variation and from the so-called 'opposition effect', which occurs at small phase angle^{14,15}. It is then possible to estimate the brightness of the illuminated side for an infinite optical thickness, with $B \approx B' \approx 26^\circ$ at any phase angle $\alpha \leq 1^\circ$. We assumed this brightness to be equal to b_E . In the simplest model (isotropic multiple scattering, no reflected light from Saturn's disk, see below), ϕ_s is approximatively a linear function of the product $B \times B'$, so that the extrapolation of $\phi = \phi_E + KBB'$ to $BB' = 0$ provides $z = \phi_E/b_E$ (Fig. 2).

Figure 2 shows the results of measurements at 20 arc s and 17 arc s from the centre of the planet (2.05 and 1.74 equatorial radii respectively). The ring seems to be systematically brighter in the outer part than in the inner part. We observe in particular a condensation of light around 21.8 arc s (ref. 10), which may be due to 'gaps' in A ring. On the other hand, the inner part is more opaque, and thus darker when transmitted light is observed. We used only the data at 17 arc s (Fig. 2b) for the determination of physical thickness z because the residual contribution of dark side at 20 arc s seems to remain significant, even near Earth's transit, on 12 March. The high value of z at $BB' = -0.0219$ (deg)² in Fig. 2b corresponds to an observation of 29 February (SATU 29.11 on Table 1) when the elevation B of the Earth was relatively large, and was probably due to the contribution of indirect illumination by Saturn's globe. This contribution is not significant at 20 arc s. These data give evidence of the reflected light from Saturn's globe, and cannot be used for a direct determination of the thickness. The vertical error bars on Fig. 2a, b are due to uncertainties in the microdensitometric measurements only. The horizontal bars are due to uncertainties in the values of B and B' , due to an inaccuracy on Saturn's pole position. There are also uncertainties in satellite magnitudes (± 0.15 mag), so that we derived from Fig. 2b:

$$z = 1.4 \pm 0.3 \text{ km}$$

Note that this is an estimate of the thickness of the model which may not be realistic. Actually, the notion of thickness itself is not evident as the rings may be warped, or surrounded by an 'atmosphere' of small particles, or by a belt of little satellites (two have already been discovered at 2.5 planetary radii whereas ring A terminates at 2.3 radii). Any of these phenomena would significantly contribute to the flux ϕ . These effects might explain the condensations observed in the rings (we

notice as well an increase in brightness from the inner to the outer part of the rings on some plates taken on 12, 15 and 16 March)¹⁰. The cross-section of the edge is probably not rectangular as in Fig. 1, but rather curved or tapered. An upper limit of 1.7 km for the 'photometric apparent thickness' can be confidently claimed. We do not yet know precisely what the quantitative consequence of the edge effect is on the brightness of the edge that is the result of the escape of light through the sides of the ring (dashed rays on Fig. 1b). At any rate, the brightness due to single-scattering only is not affected by this edge-effect. The larger the asymmetry parameter g of the particle phase function, the weaker the spreading of light through both sides. The edge effect would be more important in blue light ($g = -0.328$) than in visible light ($g = -0.386$) (refs 14, 15). Preliminary estimates⁹ seem to suggest that this effect could lead to an overestimate of b_E , and hence an underestimate of z , whose value could be 40% greater at most.

From the photographic plates of 1966, Focas and Dollfus¹⁶ derived $z = 2.5 \pm 1.4$ km, whereas Lumme and Irvine^{17,35} gave $0 < z < 2.8$ km. In 1980, better photometric accuracy of electronic receptors, sophisticated computer image analysis and larger instruments have produced a more reliable photometry. Furthermore, the conditions of observation in 1980 were better than in 1966 as the crossing of rings plane in 1980 occurred closer to opposition when the elevation B' of the Sun was smaller (0.15° and 2.75° in 1980 and 1966 respectively) so that the side S facing the Earth was darker. There was no significant observation corresponding to BB' smaller than 0.014 in 1966 and thus the extrapolation was less accurate. In particular, we can claim that the 'photometric apparent thickness' is > 1 km. The structure, the density and the particle albedo of the E ring are totally unknown. There are probably complicated structures and condensations in the equatorial plane as well as the perpendicular direction. Burns *et al.*¹⁸ have shown that a warping of the Laplace plane can account for an apparent total 'thickness' as much as a few hundred metres. The observed brightness includes non-negligible contributions of the E ring, of large chunks and condensations and of the warping of the disk. In a disk made of a continuous distribution of particles of different sizes, as is the case for Saturn's rings, the contribution of the large particles to the 'apparent photometric thickness' predominates. Thus, observations at ring-plane passage do not necessarily suggest a 'thick' ring as it is often claimed¹⁹ and are not necessarily in contradiction with a ring maintaining a thickness of only a few particles radii as suggested by the study of

systems of inelastically colliding particles^{20,21}. On the contrary, the 'theoretical thickness' can be larger than a few particles radii.

Thickness of a three-dimensional system of inelastically colliding particles

The controversy over the vertical structure of the rings (see for examples refs 22, 23), whether it is a single layer in thickness, a few layers or many layers thick, is not merely an academic exercise; it has implications for the dynamics and evolution of the rings as well as for the mean sizes and surface topographies of the ring particles^{12,20,21}.

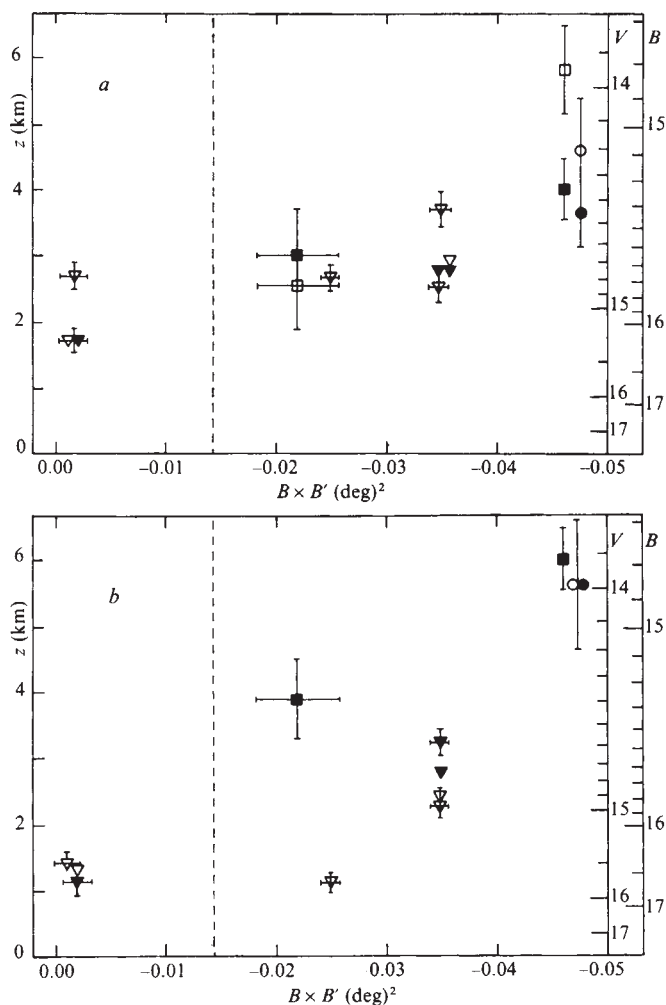


Fig. 2 Thickness ϕ_c/b_e (km) as a function of the date of observation (or of the product of the angle BB' (deg^2)). The quantity ϕ_c/b_e expressed in the text in arcs, has been converted into kilometres. On each plate two scans were made, when possible, at 20 arc s from the centre of the planet (East and West sides), and two at 17 arc s. We have so far reduced plates in blue and visible lights; the scales on the right provide the mag/arc s of the rings in these two lights. The broken line indicates the lowest value of BB' for the observations in 1966. Without data represented by points on the left, the extrapolation to obtain the apparent thickness is much more difficult.

Side	East	West
Colour		
Visible	▼	▽
Blue	●	○
Total	■	□

a, Data corresponding to a scan at 20''. *b*, Data corresponding to a scan at 17''.

It has been shown^{20,21,24} that after a very fast flattening of the order of 20 collisions per particle, a three-dimensional system of inelastically colliding particles reaches a quasi-equilibrium state in which the thickness of the newly formed disk is finite and in which collisions still occur. The combined effect of differential rotation and inelastic collisions, makes the disk spread very slowly, particles moving both inwards and outwards carrying out some angular momentum. For Saturn's rings, the time scale of flattening is of the order of a few weeks, the time scale of the quasi-equilibrium state is of the order of 10^{14} yr and the system reaches a third hypothetical collisionless state in a time scale of the order of 10^{21} yr (refs 20, 21). Although this result disagrees strongly with the time scale obtained by Jeffreys²⁵, his paper contains an erroneous calculation (see ref. 24, and M. Hénon, personal communication).

To reconcile the 'many-particle thick' conditions apparently required by recent models of ring photometric properties, which are derived from a variety of optical (opposition effect) and radio observations^{23,26,27}, Burns, Cuzzi *et al.*^{18,28,29} have considered several mechanisms as possible sources of energy for continually thickening the rings such as: gravitational perturbations by Saturnian satellites, the Sun, and the oblateness of Saturn; meteoroid bombardments; radiation pressure, and mutual gravitational scattering of particles.

We have recently studied simple models of three-dimensional systems of inelastically colliding particles in which the attraction between particles has been neglected (and so particle orbits are Keplerian around a central mass). In these models particles have the same masses and radii and the loss of energy during a collision does not depend on the relative velocity of the two colliding particles. When a large part of the orbital energy is lost after a collision, an instability phenomenon appears. For very inelastic collisions and contrary to the behaviour of the first models²¹ (Fig. 3, zone B), the disk is completely flattened and the system becomes a two-dimensional configuration in which collisions continue to occur (Fig. 3, zone A). A sharp transition L_1 separates zones A and B. A linear stability analysis of the observed behaviour has been made: differential rotation constantly feeds energy into horizontal motions and thus maintains a finite dispersion of horizontal velocities, whilst vertical motions are only a by-product of the horizontal activity and do not necessarily arise³⁰. A linear stability analysis of a flat disk of colliding particles gives a condition for stability:

$$\gamma(k-k')^2 < 4(1-k'^{-2}) \quad (1)$$

where $\gamma = \langle V_r^2 / \omega^2 r^2 \rangle v_r$ is the relative velocity of two particles, r the radius of one particle, ω the angular velocity, $1+k$ and $1-k'$

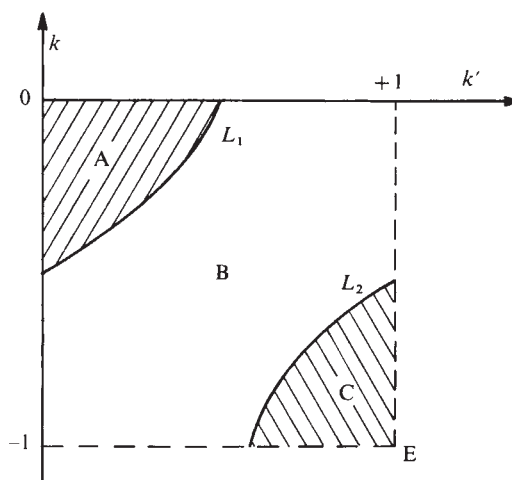


Fig. 3 The flattening of the system is a function of the rebound coefficients k and k' . In zone A, the system is completely flattened. In zone B the thickness of the disk decreases towards a value of the order of a few times the size of a particle. In zone C, the thickness of the disk increases. There exist two sharp limits L_1 and L_2 between the three zones, A, B, and C.

the relative loss of velocity after one collision in the normal and the grazing direction respectively. $(k, k') = (0, 0)$ corresponds to a complete inelastic collision and $(k, k') = (-1, 1)$ to an elastic collision. The evolution of models with velocity dependence of k, k' is essentially the same³¹. There is a second sharp transition L_2 between zone B and zone C (Fig. 3). In zone B, the thickness of the disk decreases and reaches a value of the order of r/R , where r is the radius of a particle and R some characteristic dimension of the system. In zone C, the thickness of the disk increases and the system becomes a true three-dimensional system. On the one hand, keplerian motion tends to establish an anisotropic distribution of velocities with a velocity twice as large in the radial direction than in the transverse direction. On the other hand, collisions tend to establish an isotropic distribution of velocities. If the degree of inelasticity is larger (Fig. 3, zones A and B), a smaller quantity of energy is extracted from the ordered motion and the orbits become more and more circular and co-planar. A crude calculation of the equilibrium between these two phenomena gives the equation:

$$L_2: k^2 + k'^2 = 1.28 \quad (2)$$

(For L_2 see ref. 31.) Zone C probably does not correspond to the Saturn case, unless nonlinear effects damp the vertical motions when the system becomes very thick.

In a real system, most collisions would be between particles of very unequal size³². We shall give here only those results concerning the mutual evolution of two different sized particles. Generally, the two groups of particles evolve in essentially the same way. There is no real equipartition of energy, but rather a kind of separation. The mean inclination and the mean eccentricity of the large particles is smaller than the mean inclination and the mean eccentricity of the small particles (Fig. 4). The evolution is essentially similar to that of the first models. The more elastic the collisions, the bigger the separation; the larger the mass ratio, the bigger the separation; there would not seem to be any separation in the plane of the disk. One can see numerically and by a crude calculation³³ that the mean velocity $\langle v_1 \rangle$ of the large particles is proportional to the mean velocity $\langle v_s \rangle$ of the small particles:

$$\langle v_s \rangle \propto \frac{1}{(1 - k^2)^{1/2}} \langle v_1 \rangle \quad (3)$$

Generally the massive particles evolve faster than the lighter ones. The relation ($ar^2 < 3 \times 10^{10} \text{ yr m}^2$) between the size r of the particles and the age a of the system, shown—for systems of identical particles²¹, remains valid for each component individually. To the extent that Saturn's rings may be modelled in this way, then if the rings are as old as the Solar System, the maximum size of the particles is 2.5 m. A system made essentially of large particles, would presumably have been created much later. Cuzzi *et al.*²⁹ have shown that the ring systems attains a steady state in which gravitational scattering (not considered here) by the large particles impacts random velocity components to the small particles while inelastic collisions (not quantitatively treated in the Cuzzi's model) decrease these random velocities.

Received 1 September; accepted 18 November 1980.

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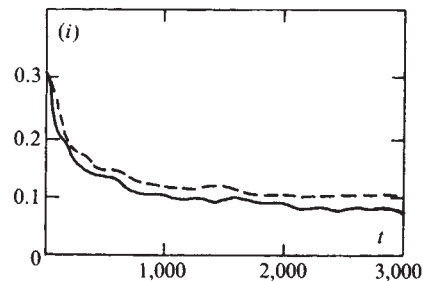


Fig. 4 Variations as a function of time of the mean inclination of the particles (which is a good measure of the flattening of the system). The results for large particles are shown by a solid line and those for small particles are given by the dashed line. The particles have the same density.

We have studied systems in which a satellite orbits around the primary on an orbit inclined with respect to a disk of colliding particles. The influence of the satellite on the thickness is negligible. Detailed results on small changes in the mean plane's location due to the action of the satellite are given elsewhere³⁴.

Conclusion

The main results of our numerical simulation are:

- (1) In the case of almost elastic collisions, a disk of colliding particles can expand vertically to become a three-dimensional system.
- (2) When particles of different masses and sizes collide there is no real equipartition of energy, but rather a kind of separation between the different populations.
- (3) Satellite perturbations are not very efficient in thickening a disk of colliding particles.

The 'local' thickness of the rings is probably of the order of a few particles radii or some tens of metres²¹. Unless some as yet not understood mechanism reduces the vertical motions, the phenomenon observed in zone C of Fig. 3 is apparently not present in Saturn's rings, as it would lead to a much larger thickness. Contrary to what one could deduce from a relative velocity of the order of only $10^{-2} \text{ cm s}^{-1}$ for 2-m sized colliding particles in Saturn's rings, there is enough energy lost during the collisions. If not the rings would be much thicker. On a large scale, the rings are probably slightly warped¹⁸ and are possibly surrounded by an atmosphere¹⁹ and by small satellites or large chunks of matter just outside (as discovered in March 1980)^{4,5} or even inside the rings. This would produce on average an apparent edge-on thickness of several hundred metres and would make the true physical thickness (a local vertical scale height of the rings) impossible to determine from ring-passage observations from the Earth or from fly-bys.

We thank M. Hénon for helpful advice on the theoretical studies; J. Lecacheux, P. Laques, R. Despiou, G. Wlérick, B. Servan, D. Michet, L. Renard, and A. Bijaoui for observations and assistance, and A. Cazenave, J. Burns and R. Greenberg for stimulating discussions. Much of this work was supported by the RCP 544 'Anneaux' and the ATP 'Planétologie' of the CNRS.

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New data base for climate studies

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Until the advent of satellite data it had been impractical to measure continental snow cover. Beginning with the ESSA 3 satellite in late 1966, and continuing to the present NOAA and GOES series satellites, Northern Hemisphere continental snow cover has been routinely mapped by the National Oceanic and Atmospheric Administration (NOAA). This mapping has produced a reliable, timely and relatively unbiased continental snow-cover data base for use in climate studies.

In recent years the impact of global snow and ice cover on climatic processes has received increased attention¹⁻⁵. Kukla⁶ stated that "to understand properly the mechanisms of weather and climate variability we need to understand the variability of snow and pack-ice fields". The Global Atmospheric Research Program (GARP) has recommended that sensitivity studies of the climatic effects of global and regional anomalies of snow and ice cover be performed with various types of general circulation models⁷. Specifically, continental snow cover has important climatic impacts on the radiation balance, surface and air temperature, cloudiness, soil moisture, water storage and precipitation. Continental snow cover can also have an impact on energy requirements, transportation systems and food supplies.

Because of the climatic-economic importance of continental snow cover, GARP⁷, the Committee on Space Research (COSPAR)⁸, and the United States Climate Program Plan⁹, have recommended that global monitoring of the extent of snow cover be included in both short- and long-term observational programmes. Until 1966, however, the monitoring of the areal extent of continental snow cover was limited to point measurements and extrapolation between these often widely separated

points. Mountainous and sparsely inhabited areas were poorly represented in this type of monitoring. In November 1966 the first satellite-derived Northern Hemisphere Weekly Snow and Ice Cover Chart was produced by the National Environmental Satellite Service (NESS) of NOAA. Since then, continental snow-cover extent has been monitored on a weekly basis by satellites. In this article we demonstrate the ability of satellites to monitor an important climatic parameter and discuss the 14-yr satellite record of continental snow cover.

The NOAA/NESS satellite capability

The NOAA/NESS satellites and sensors used in mapping Northern Hemisphere continental snow cover are listed in Table 1. Note that the high-resolution VHRR, AVHRR and VISSR data are primarily available only for North America. Table 1 shows that satellite data were available at all times from late 1966 to the present for monitoring continental snow cover. Of course, the snow-cover data have come from a variety of satellites and sensors, all subject to instrumental variation, degradation and drift. The impact of these factors on mapping continental snow cover, however, has been negligible.

Table 1 NOAA/NESS satellites and sensors used in mapping Northern Hemisphere snow and ice cover

Satellite	Sensor*	Spectral band (μm)	Subpoint resolution (km)	Period of operations
ESSA 3	AVCS	0.5-0.75	3.7	2 October 1966-9 October 1968
ESSA 4	APT	0.5-0.75	3.7	26 January 1967-6 December 1967
ESSA 7	AVCS	0.5-0.75	3.7	15 August 1968-19 July 1969
ESSA 8	APT	0.5-0.75	3.7	15 December 1968-12 March 1976
ESSA 9	AVCS	0.5-0.75	3.7	26 February 1969-15 December 1973
ITOS 1	AVCS	0.5-0.75	3.7	23 January 1970-17 June 1971
	APT	0.5-0.75	3.7	
	SR	0.52-0.73	3.7	
		10.5-12.5	7.4	
NOAA 2	VHRR	0.6-0.75	1-1.9	15 October 1972-30 January 1975
		10.5-12.5	1-1.9	
	SR	0.52-0.73	3.7	
		0.50-0.94	3.7	
NOAA 3	VHRR	0.6-0.75	1-1.9	6 October 1973-17 December 1974
		10.5-12.5	7.4	
	SR	0.52-0.73	3.7	
		10.5-12.5	7.4	
NOAA 4	VHRR	Same as NOAA-3		15 November 1974-15 September 1976
	SR	Same as NOAA-3		
NOAA 5	VHRR	0.6-0.75	1-1.9	29 July 1976-1 March 1979
		10.5-12.5	1-1.9	
	SR	0.52-0.73	3.7	
		0.50-0.94	3.7	
		10.5-12.5	7.4	
TIROS N	AVHRR	0.55-0.68	1	6 November 1978-20 January 1980
		0.725-1.10	1	
		3.55-3.93	1	
		10.5-11.5	1	
NOAA 6	AVHRR	Same as TIROS-N		16 July 1979-Present
GOES series	VISSR	0.55-0.70	1-7.4	17 May 1975-Present
		10.5-12.5	7.4-14.8	

* Cameras and sensors: AVCS, advanced vidicon camera system; APT, automatic picture transmission; SR, scanning radiometer; VHRR, very high resolution radiometer; AVHRR, advanced very high resolution radiometer; VISSR, visible and IR spin scan radiometer.

The NOAA/NESS Northern Hemisphere snow-cover data base

Since November 1966, using the visual band sensors listed in Table 1, the Synoptic Analysis Branch (SAB) of NESS has prepared the Northern Hemisphere Weekly Snow and Ice Cover Chart (Fig. 1). These charts, at a scale of 1:62,500,000, show the areal extent and brightness of continental snow cover but do not indicate snow depth. The preparation of the charts and the definition of terms used in the analysis have been

described in detail in an unpublished report by SAB; we give here only brief details.

Early in the week the analyst, a satellite meteorologist, makes a pencil trace of the previous week's chart. Daily he collects all of the visual band data received during the previous day, compares snow and ice cover interpreted from the imagery with snow cover on the previous week's chart, and makes changes. Each area of the new chart is therefore the latest cloud-free snow observation of that particular area of the world. If an area is cloud covered for several days, the analysis of snow cover for

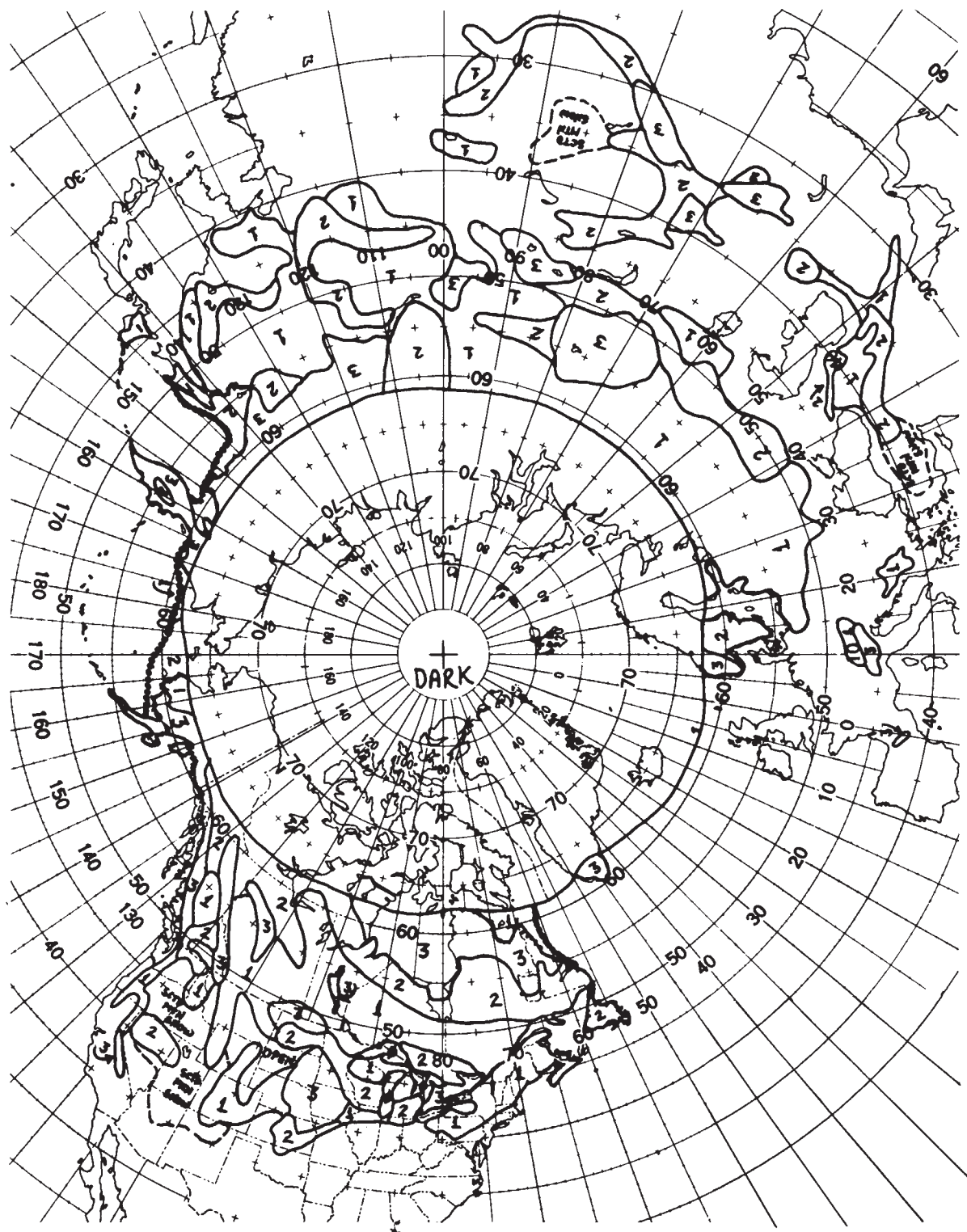


Fig. 1 Typical snow and ice chart of the Northern Hemisphere for the period 29 December 1974 to 4 January 1975 (scale 1:62,500,000), prepared by the Synoptic Analysis Branch, NOAA/NESS. Reflectivities: 1, lowest; 2, moderate; 3, highest. —, snow; ○, ice. Note the 'dark' area where visible data cannot be collected during the polar winter.

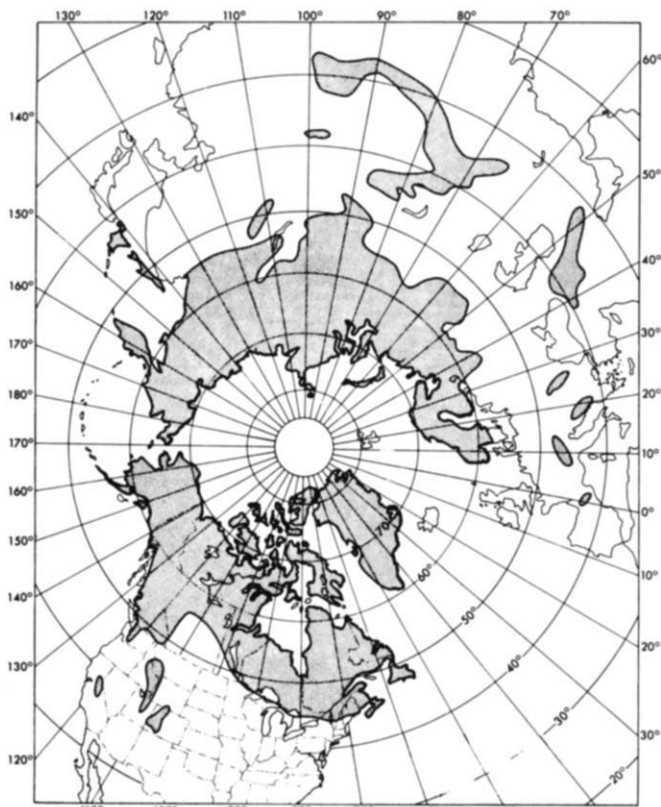


Fig. 2 Monthly mean snow cover chart of the Northern Hemisphere for April 1978 on a stereopolar projection at a ~1:100,000,000 scale. This chart was prepared from the weekly charts.

that area will necessarily be several days old. If the area remains cloud covered for the entire week, the previous week's analysis is carried over to the new chart. Finally, the analyst compares his chart with the surface synoptic reports to check for errors or to confirm drastic changes in snow cover, as can result from a new snowfall or rapid snow melt. At the end of the week the new chart is rendered in final form and transmitted to the user community. Solid lines on the chart indicate boundaries between areas of substantially differing brightness (reflectance) of the snow field. The chart is divided into three categories of brightness: (1) lowest reflectivity; (2) moderate reflectivity; and (3) highest reflectivity. These categories are subjective and are chosen by the analyst. Guidelines aiding the analyst in assigning these reflectivity classes include the following. First, the designated reflectivity of a given snow area is the average reflectivity within that boundary, that is, a category (2) could be the average of a small area of category (3) and a small area of category (1), or the entire area could be a category (2) reflectivity. Second, the reflectivity of categories is relative to their surroundings at a particular time of year. For example, although it is much less bright in the winter due to reduced solar illumination, the Greenland icecap is labelled category (3) in both summer and winter. It is, however, much brighter than its surroundings even at this time. Third, when snow-covered mountain tops are seen in large open areas that average less than category (1), the area is labelled as scattered mountain snow. Fourth, when the distinction in reflectivity between small areas of land and water is great, such as over Arctic Canada in early summer, the entire area is labelled 3 over water and 1 over land.

In winter much of the far northern latitudes lies in total darkness or near darkness. Consequently, the visual-band sensors on the satellite are inoperative, and areas around the pole are shown as 'dark'. At the winter solstice the snow and ice chart is labelled 'dark' north of about 55°N.

The quality of the weekly charts is affected by several factors: The charts are based on interpretations by a variety of observers,

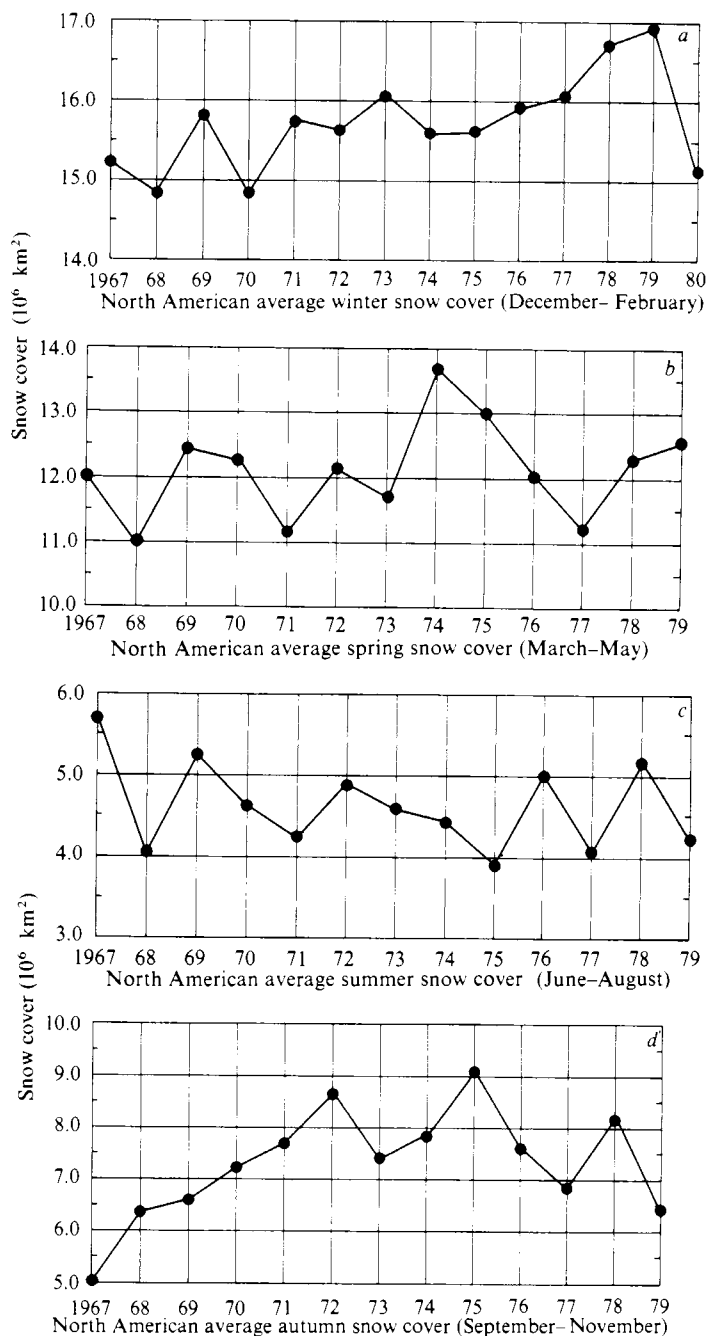


Fig. 3 a, Average North American winter snow cover for the period 1967-1980. Calendar year notation is such that 79 represents the winter of 1978-79. b, Average North American spring snow cover for the period 1967-79. c, Average North American summer snow cover for the period 1967-79. d, Average North American autumn snow cover for the period 1967-79.

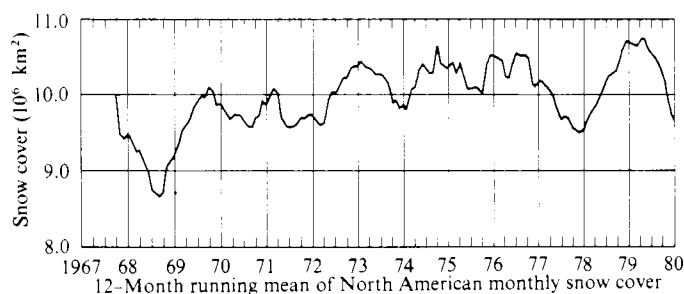


Fig. 4 Twelve-month running mean of North American monthly snow cover. The data are plotted on the last month of the 12-month period.

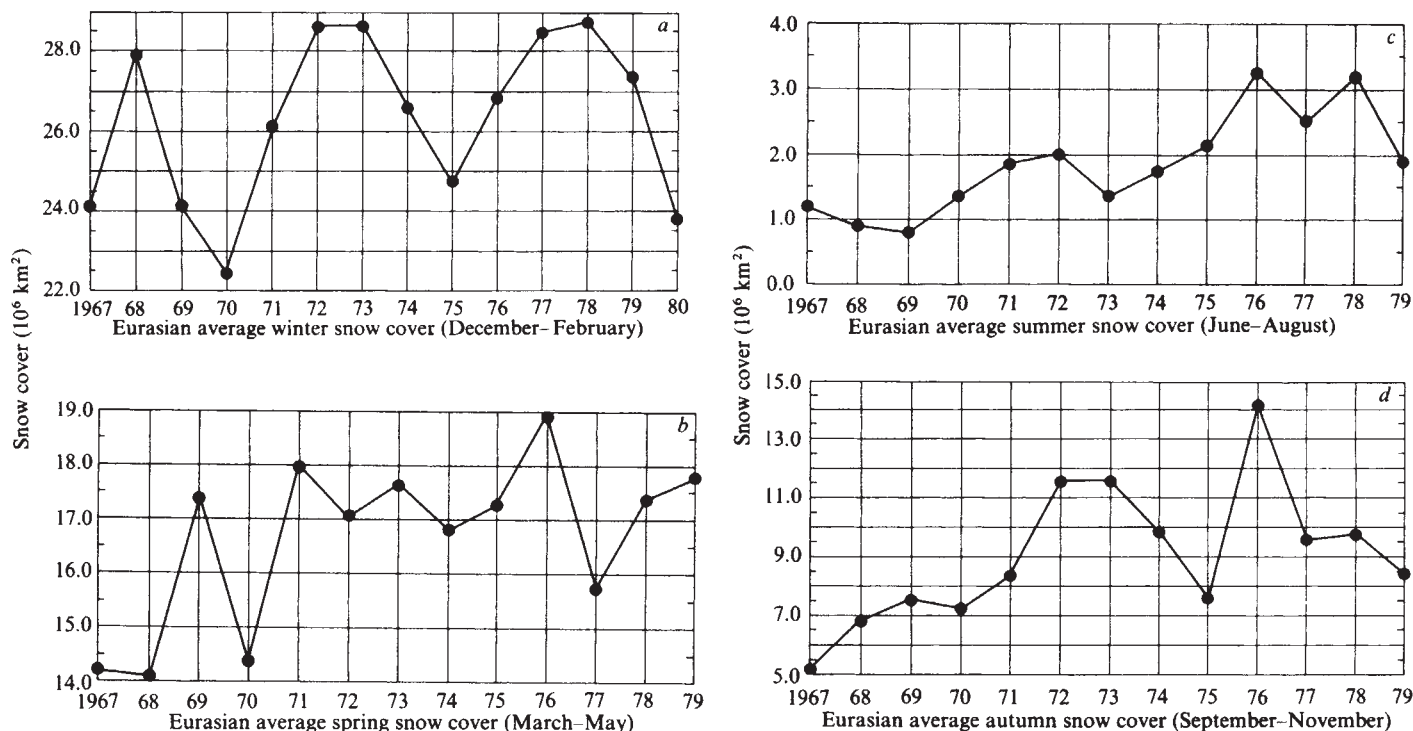


Fig. 5 a, Average Eurasian winter snow cover for the period 1967–80. Calendar year notation is such that 79 represents the winter of 1978–79. b, Average Eurasian spring snow cover for the period 1967–79. c, Average Eurasian summer snow cover for the period 1967–79. d, Average Eurasian autumn snow cover for the period 1967–79.

thus operator bias is undoubtedly present; the scale of the charts limits the precision of snow line positioning to about 20–50 km (ref. 10); Himalayan snow cover was not consistently mapped during the 1966–74 period; the charts show three levels of increasing improvement in consistency and detail—1966–70, 1971–73, and 1974 to the present. Kukla and Robinson¹¹ analysed the accuracy of the NOAA/NESS weekly charts by comparing them with an independent daily analysis of weekly snow cover based on satellite data and ground station reports for the United States. The analysis covered periods for November 1975, February 1977 and February 1978. After measuring the areal extent of snow cover derived from both the NOAA/NESS and independent analysis, they concluded that: the areal differences tend to be smaller in the more recent charts; the average areal differences are usually less than 10%; the accuracy of the NOAA/NESS weekly charts is adequate for climate-related studies on a continental or hemispheric scale.

Monthly mean snow-cover charts (Fig. 2) have been constructed from the weekly charts for all months from November 1966. The winter (December to March) monthly snow-cover maps from 1966 to 1976 have been published¹² and a digitized atlas of all of the monthly maps is being prepared. The monthly

charts are constructed by deriving a subjective average of the NOAA/NESS weekly chart boundaries of each month. The areal extent of continental snow cover within this average monthly snow-cover boundary is then measured and recorded. All continental areas within the 'dark' zone are assumed to be completely snow-covered and are measured as such. The snow-cover values are corrected to represent true surface area coverage. The error in the value of areal snow cover owing to the analogue averaging of weekly data to derive the monthly snow line is approximately 2–5% and randomly distributed².

A look at the record

Figure 3 shows a graphical record of North American seasonal snow cover from 1967 to 1980. The average winter snow cover (Fig. 3a) rose steadily from 1975 to 1979—an 8% increase when 1979 is compared with 1975. The winters of 1977, 1978 and 1979 are discussed in more detail elsewhere^{13–15}. During the severe North American winter of 1978 nearly 70% of the continent was snow-covered. Although characterized by marked fluctuations, spring (Fig. 3b) and summer (Fig. 3c) average snow cover has been around $12.1 \times 10^6 \text{ km}^2$ and $4.6 \times 10^6 \text{ km}^2$, respectively. Autumn average snow cover (Fig. 3d) shows two distinct periods: a period of increase from 1967 to 1972 and a period of levelling off with sharp annual fluctuations from 1972 to 1978. Figure 4 is a 12-month running mean (to remove the annual cycle) of North American monthly snow cover and it shows a slight trend towards increasing snow cover for the continent.

As Eurasia is a larger continent than North America, Eurasian seasonal snow-cover fluctuations exhibit a wider range of values, as revealed by Fig. 5. Winter snow cover (Fig. 5a) has an interesting cyclic pattern but the data set is too short to confirm the apparent repeatability. Very large areas of the continent were covered by snow during the winters of 1972, 1973, 1977 and 1978. At these times over 50% of the Eurasian land mass was snow-covered. Spring snow cover (Fig. 5b) varies markedly, showing especially large annual changes from 1968 to

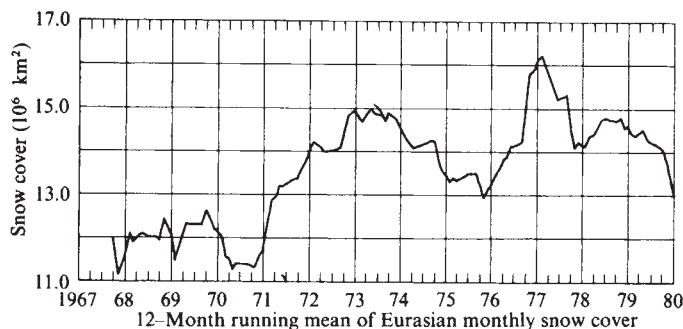


Fig. 6 Twelve-month running mean of Eurasian monthly mean snow cover. The data are plotted on the last month of the 12-month period.

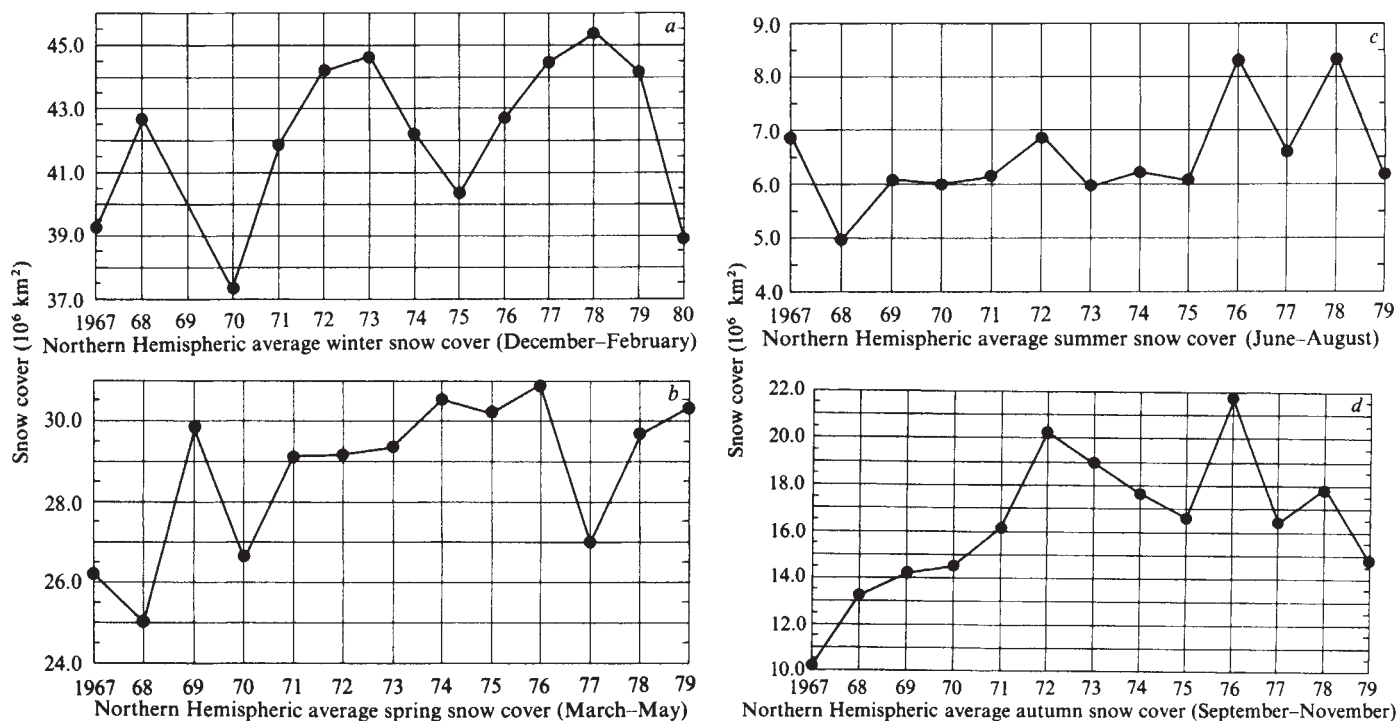


Fig. 7 *a*, Average Northern Hemisphere winter snow cover for the period 1967–80. Calendar year notation is such that 79 represents the winter of 1978–79. *b*, Average Northern Hemisphere spring snow cover for the period 1967–79. *c*, Average Northern Hemisphere summer snow cover for the period 1967–79. *d*, Average Northern Hemisphere autumn snow cover for the period 1967–79.

1971 and 1975 to 1978. Although Eurasian summer snow cover (Fig. 5c) shows an increasing trend, the reality of that trend is dubious because of the previously mentioned inconsistency in mapping Himalayan snow cover during the 1966–74 period. The average of the summer snow-cover values from 1975 to 1979, $2.6 \times 10^6 \text{ km}^2$, is probably a more realistic value than the earlier summer values. Autumn snow cover (Fig. 5d) also shows an increasing trend over the whole satellite record with a particularly sharp increase in 1976. This increase was in a large part due to a satellite-based record high October snow cover of $19.7 \times 10^6 \text{ km}^2$ compared with a 10-yr (1967–76) average of $9.6 \times 10^6 \text{ km}^2$. Most of this record snow cover occurred from 11 to 17 October when the snow line advanced from 60 to 50°N in an area longitudinally bordered by 30°E and 80°E. This area represents more than $3 \times 10^6 \text{ km}^2$ of snow cover, all in a 1-week period. The snow cover certainly contributed to the high (45%) satellite-derived October zonal albedo value for the area 0–117.5°E and 60–50°N (ref. 16). Once established the snow cover persisted throughout the autumn and into the winter. The effect of this anomalous and sudden snow cover on the radiation

balance and atmospheric circulation would be interesting for researchers concerned with climate feedback mechanisms.

The Eurasian 12-month running mean of monthly snow cover is shown in Fig. 6. During the satellite record Eurasia has experienced two periods of large increases in snow-cover extent, one from 1971 to 1973, the other from 1976 to 1977. The running mean for Eurasia also shows an overall upward trend.

Except for summer, the proportionally larger variations in Eurasian snow cover dominate the North American variations. The result is that trends in the Northern Hemisphere and Eurasian data for the winter, spring and autumn are similar (Fig. 7a, b, d). During the summer season most of the Northern Hemisphere snow cover is in Greenland and the Canadian archipelago, as shown by the similarities in the graphs of Fig. 3c and Fig. 7c. The 12-month running mean of Northern Hemisphere snow cover (Fig. 8) is also dominated by the Eurasian snow cover (Fig. 6), both showing similar trends and fluctuations. From these similarities one may conclude that in any study of hemispheric climatic feedback mechanisms involving snow cover, Eurasia has a greater feedback potential than North America.

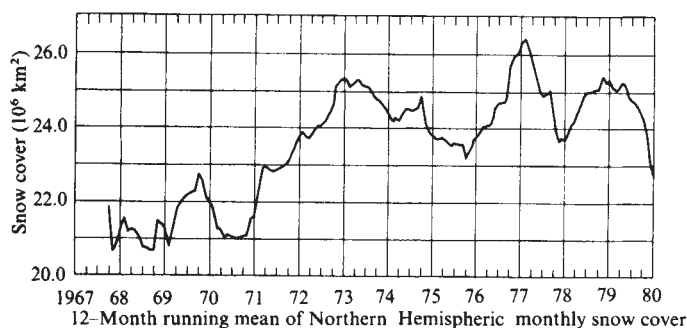


Fig. 8 Twelve-month running mean of Northern Hemisphere monthly mean snow cover. The data are plotted on the last month of the 12-month period.

Conclusions

The present satellite-derived data provide a rapid and timely synthesis of global snow-cover information to assess current conditions. This accumulation of a reliable and relatively unbiased continental snow-cover climatic data base has application in seasonal weather forecasting and in global and regional modelling. As the satellite snow-cover record is extended temporally, it should provide a new understanding of the dynamics of global and regional climate.

We thank Paul McClain for his review, Dennis Haller of SAB for the unpublished description of the construction of the Northern Hemisphere Weekly Snow and Ice Cover Charts, George Kukla for his continued support, Craig Berg for his analysis of the snow cover data during 1978–79, and Mrs Olivia Smith for assistance.

Received 14 July; accepted 13 November 1980.

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Identification of a glycophorin-like molecule at the cell surface of rat thymocytes

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There are three predominant glycoproteins in the rat thymocyte plasma membrane. Two of these have carbohydrate compositions that are characteristic of structures N-glycosidically linked to protein. The other glycoprotein is very different, having about 20 O-glycosidically linked carbohydrate units per 100 amino acids. It has similarities to the major sialoglycoprotein of human erythrocytes.

CELL-SURFACE glycoproteins with large amounts of carbohydrate are found on most eukaryotic cell types. Although little is known of the structure and functions of these molecules, it is widely thought that they may be involved in determining interactions between cells^{1,2}. We have now completed the initial characterization of the predominant glycoproteins of rat lymphoid cells.

Much of the membrane carbohydrate on rodent lymphoid cells is found on a small number of cell-surface glycoproteins. These can be identified on intact cells by labelling sialic acid residues with ³H-borohydride after mild periodate oxidation³. In the rat and mouse the glycoproteins so labelled are very similar in apparent molecular weight (MW) when analysed by SDS-polyacrylamide gel electrophoresis (PAGE)^{4,5}. Figure 1 shows the patterns for rat T and B lymphocytes and thymocytes. T lymphocytes have bands of 170,000 and 95,000 apparent MW (Fig. 1, track 1) and B lymphocytes have one major band of 200,000 apparent MW (Fig. 1, track 2). Thymocytes have predominant bands of 150,000, 95,000 and 25,000 apparent MW (Fig. 1, track 3). The periodic acid Schiff (PAS) staining method identifies similar glycoprotein bands in lymphoid cell membranes analysed by SDS-PAGE⁶.

Conventional or monoclonal antibodies against these glycoproteins were prepared and used for analysis and purification of the antigens. The 25,000 apparent MW glycoprotein of thymocytes is the Thy-1 antigen⁷. This molecule is an abundant glycoprotein of rodent thymus (10⁶ molecules per thymocyte) and brain and occurs in two allotypic forms in the mouse (reviewed in ref. 8). The correct MW of thymocyte Thy-1 is 18,700 (ref. 9), of which 30% is carbohydrate. The polypeptide of rat brain Thy-1 has been sequenced and the attachment points for three N-linked carbohydrate structures have been identified¹⁰. The Thy-1 antigen seems to be structurally homologous to an immunoglobulin domain^{11,12}.

The 150,000, 170,000 and 200,000 apparent MW glycoproteins of thymocytes, T cells and B cells share antigenic determinants. These glycoproteins seem to be specific to haematopoietic cells and are synonymous with the leukocyte-

common (L-C) antigen in the rat¹³ and the T200 glycoprotein in the mouse¹⁴. In both rat and mouse these molecules bear allotypic determinants – the Ly-5 mouse alloantigen is the T200 glycoprotein¹⁵ and the ART and Ly-1 rat alloantigens are the L-C antigen¹⁶. The rat thymocyte L-C antigen has been purified using a monoclonal antibody affinity column. There are about 70,000 molecules of antigen per thymocyte¹³.

The 95,000 apparent MW bands found on rat thymocytes and T cells share an antigenic determinant recognized by a mouse anti-rat monoclonal antibody called W3/13 (ref. 5). This antibody was prepared after immunization of mice with rat thymocyte plasma membrane¹⁷. The W3/13 antigen is found on thymocytes, T (but not B) lymphocytes, bone marrow neutrophils and brain, and also on plasma and myeloma cells (ref. 17 and D. Secher, personal communication). The antigen from haematopoietic cells is in every case a glycoprotein of apparent MW 95,000 which is heavily labelled by NaB³H₄ after mild periodate oxidation (ref. 5 and W.R.A.B., unpublished data for rat myeloma cells). Brain W3/13 antigen has not been characterized. The 95,000 MW glycoprotein is notable because it does not bind at all to lentil lectin^{5,6} and also because removal of sialic acid with neuraminidase results in an apparent increase in size on SDS-PAGE. The asialo glycoprotein can be labelled with ³H-borohydride after oxidation of galactose with galactose oxidase⁴. The results for rat thymocytes are shown in Fig. 1, track 6. The bands of apparent MW 150,000 and 25,000 are unaffected by neuraminidase treatment but the 95,000 apparent MW band is found at 105,000 apparent MW after removal of sialic acid (compare tracks 3 and 6 in Fig. 1). Both these forms of the molecule bind to affinity columns of W3/13 antibody attached to Sepharose 4B (Fig. 1, tracks 5 and 8). Note that not all of the 95,000 or 105,000 MW bands bind to the antibody column (Fig. 1, tracks 4 and 7); we will discuss this further below.

This paper reports the purification of the W3/13 (95,000 MW) glycoprotein and the amino acid and carbohydrate compositions of this molecule and also of the L-C antigen (glycoprotein 150,000 MW) from rat thymocytes. The compositions are compared with those previously published for rat thymocyte Thy-1 antigen (glycoprotein 25,000 MW)¹⁸ and for glycophorin A, the major sialoglycoprotein of human erythrocytes¹⁹.

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Table 1 Purification of thymocyte W3/13 antigen

Fraction	Antigenic activity (units $\times 10^{-4}$)	Protein (mg)	Relative specific activity	Yield of antigenic activity (%)
Cells (2.82×10^{11}) + Brij 96	9.7	4,825	1	100
Brij 96 extract	9.7	2,594	1.9	100
Brij 96-deoxycholate extract	9.0	1,979	2.3	93
Sephacryl 4B-W3/13 antibody unbound fraction	0	1,802	0	0
Sephacryl 4B-W3/13 antibody bound and eluted fraction (1st)	7.6	—	—	78
Sephacryl 4B-W3/13 antibody bound and eluted fraction (2nd)	7.2	—	—	74
After Sephacryl S300 chromatography	3.7	—	—	38
After ethanol precipitation	2.8	0.198	7,000	29

Thymocytes from Sprague-Dawley rats (OLAC 76) were teased into Dulbecco's A and B buffer, pH 8.4. To 2.82×10^{11} cells in 170 ml were added 73 ml of 10% Brij 96 (Sigma), 10 mM iodoacetamide (IAA) and 10 mM diisopropyl fluorophosphate (DFP) in Dulbecco's A and B buffer, pH 8.4. The mixture was stirred on ice for 30 min, homogenized with six strokes of a Potter-Elvehjem homogenizer and then centrifuged at 6,300g for 20 min. The supernatant (205 ml, Brij 96 extract) was added to 102 ml of 3% sodium deoxycholate and 10 mM Tris-HCl, pH 8.0, and the mixture was centrifuged at 70,000g for 45 min. The supernatant (285 ml, Brij 96 sodium deoxycholate extract) was then passed at a flow rate of about 20 ml h^{-1} through a control column of mouse immunoglobulin coupled to Sepharose 4B and an 8-ml affinity column of 120 mg of the W3/13 antibody coupled to Sepharose 4B (refs 38, 39). After washing with 2% sodium deoxycholate, 10 mM Tris-HCl, 2 mM IAA and 2 mM DFP, the affinity column was eluted with 50 mM diethylamine-0.5% sodium deoxycholate, pH 11.5. The eluate was brought to pH 8.6 by the addition of glycine. This eluate was not pure W3/13 antigen and so was passed through the affinity column as above. The material specifically bound and eluted from the column a second time was then concentrated by ultrafiltration over an Amicon PM 10 membrane and chromatographed on Sephacryl S300 in 0.5% sodium deoxycholate and 10 mM Tris-HCl, pH 8.4. The eluted material containing the W3/13 antigen was concentrated before precipitation with 75% ethanol¹⁸ at $-20^{\circ}C$. One unit of antigenic activity is the amount of antigen needed to give 50% inhibition of the indirect radioactive binding assay for W3/13 (ref. 17). Protein was assayed by the Lowry method⁴⁰ for detergent extracts and by amino acid analysis for the pure glycoprotein. All procedures were carried out at $4^{\circ}C$.

Purification of the W3/13 antigen (glycoprotein 95,000 MW)

The W3/13 antigen was purified from rat thymocytes using a monoclonal antibody affinity column. In previous purifications of the Thy-1 (ref. 7) and L-C (ref. 13) antigens a plasma membrane fraction was first prepared and then solubilized in deoxycholate to give an extract which was passed through an antibody affinity column. Bound antigen was eluted from the column with high pH. This scheme posed problems with the W3/13 antigen because the glycoprotein was very susceptible to proteolysis, yielding a form with an apparent MW of 82,000 as shown in Fig. 1, track 9. This degradation occurred extensively when membranes were prepared using the Tween 40 method⁶ even though neither Thy-1 nor L-C antigen showed autolysis during this procedure. The addition of iodoacetamide and diisopropyl fluorophosphate minimized this degradation (Fig. 1, track 10) and we also found that direct solubilization of membrane from whole cells with the non-ionic detergent Brij 96 gave less autolysis. This step also resulted in the recovery of more antigenic activity than when membranes were prepared. The only disadvantage of using the non-ionic detergent was that in antibody affinity chromatography more non-antigenic proteins were adsorbed to the columns than when deoxycholate was used. To minimize this, deoxycholate was added to the Brij 96 extract and the affinity column step was carried out twice.

Table 1 legend describes the purification of the W3/13 antigen. All the antigenic activity was bound by the column and 85% of the applied activity was recovered in the high pH eluate. After repeating the affinity column step and carrying out gel filtration, about 30% of the original antigenic activity was recovered at a final purification of $\sim 7,000$ -fold. It is most unlikely that such a purification could have been achieved by any known procedure other than antibody affinity chromatography.

The purification factor is of the right order of magnitude for obtaining pure W3/13 antigen. If it is assumed that the whole

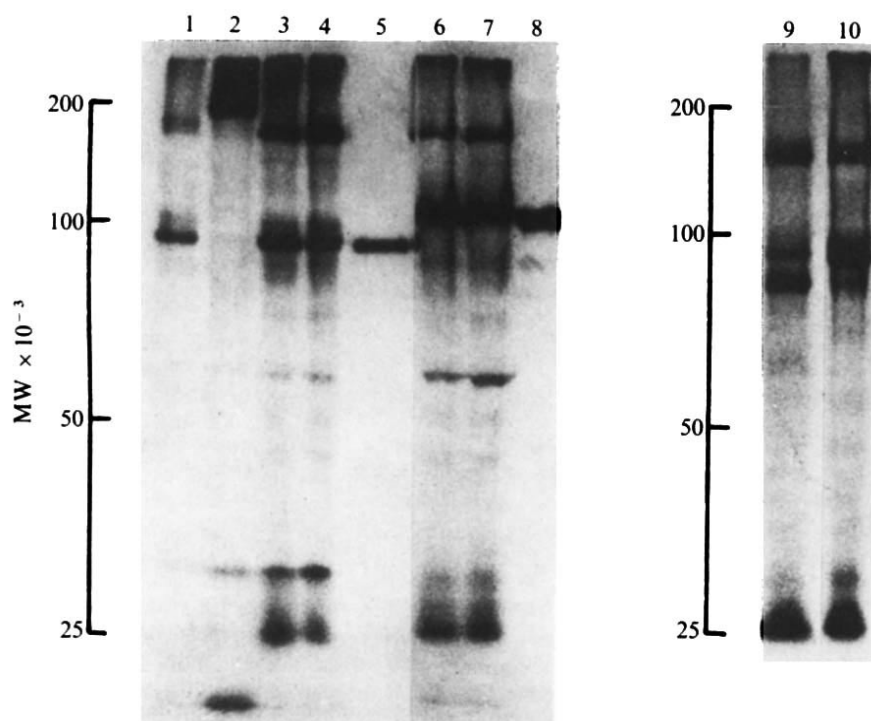


Fig. 1 SDS-PAGE of radiolabelled cell-surface glycoproteins. Tracks 1-5: glycoproteins labelled in sialic acid residues (see below). Track 1, from T lymphocytes; track 2, from B lymphocytes; track 3, from thymocytes; track 4, from thymocytes passed through a W3/13 antibody affinity column; track 5, W3/13 antigen eluted from affinity column. Tracks 6-8: glycoproteins labelled in galactose residues (see below). Track 6, from thymocytes; track 7, from thymocytes passed through a W3/13 antibody affinity column; track 8, W3/13 antigen eluted from affinity column. Track 9: thymocyte glycoproteins labelled in sialic acid from plasma membranes without inhibitors of proteolysis. Track 10: as for 9 plus inhibitors (see below). For tracks 1-8 thymocytes and T and B lymphocytes were prepared as in ref. 5 and labelled with NaB^3H_4 after oxidation with periodate³ or galactose oxidase following neuraminidase digestion⁴. Extracts were prepared by resuspending the labelled cells at 3×10^6 ml^{-1} in 3% Brij 96 in Dulbecco's A and B buffer, 5 mM iodoacetamide and 5 mM DFP. After incubation for 15 min on ice nuclei were removed by centrifugation for 10 min at 2,000g. One vol of 3% sodium deoxycholate, 10 mM Tris-HCl, pH 8, was then added to 2 vol of the Brij 96 extract. For affinity chromatography the extract was passed through a column of W3/13 antibody and washed and eluted as in Table 1. The eluted material was brought to pH 8.6 with glycine and inhibitors of proteolysis were added. For tracks 9 and 10 plasma membrane was prepared from radiolabelled thymocytes using the Tween 40 method⁶. To minimize losses $\sim 13.5 \times 10^8$ unlabelled thymocytes were added to 1.5×10^8 labelled thymocytes. For track 10 but not 9 inhibitors of proteolysis (as above) were added at each step. For all samples, glycoproteins were precipitated with 80% acetone at $-20^{\circ}C$ overnight and the precipitate was recovered by centrifugation and suspended in 8 M urea, 5% SDS, 2% dithiothreitol. The samples were boiled for 5 min and run on 10% polyacrylamide gels⁴⁶. The radioactive bands were visualized by fluorography⁵.

molecule has a MW of 95,000, then that of the protein part is 38,000 (60% is carbohydrate, see below). From this, and the data in Table 1, it can be calculated that there should be 38,000 molecules of W3/13 antigen per thymocyte. At saturation, 35,000 molecules of the W3/13 monoclonal antibody bind per thymocyte¹⁷ and this probably underestimates the number of antigenic sites by about 50% (ref. 20). Although both estimates of the amount of antigen suffer from some uncertainties, the data strongly suggest that the purified molecule is the W3/13 antigen.

The purified W3/13 antigen was analysed by SDS-PAGE followed by staining for protein with Coomassie blue (Fig. 2a) and carbohydrate with PAS stain (Fig. 2b). The purified glycoprotein stains very heavily with PAS stain and this is illustrated in Fig. 2 by comparing the staining of W3/13 antigen with that of rat brain Thy-1 antigen. With Coomassie blue stain 4 µg of protein gave roughly equal staining with both glycoproteins (Fig. 2a), whereas with PAS stain the W3/13 glycoprotein was stained much more intensely than Thy-1 (Fig. 2b). The purified W3/13 antigen showed more than one band with both Coomassie blue and PAS stain. Densitometry on the trace in Fig. 2a gave 70% of the stained material in the peak at 95,000

Table 2 Amino acid and carbohydrate composition of the W3/13, L-C and Thy-1 antigens and of human glycophorin A

Residue	Amino acids per 100 amino acid residues			
	W3/13	L-C	Thy-1	Glycophorin A
Asx	5.3	11.1	12.6	6.1
Glx	9.4	12.0	9.1	10.7
His	0.6	2.3	3.8	3.8
Lys	3.6	7.3	7.2	3.8
Arg	3.5	4.9	7.5	4.5
Thr	13.7	6.5	8.6	11.5
Ser	13.8	7.7	7.1	14.5
Pro	9.4	4.8	3.8	7.6
Ala	8.7	5.7	2.9	4.6
Cys	0	2.1	3.2	0
Gly	9.5	6.4	4.9	4.6
Tyr	0.5	3.8	2.0	3.1
Val	7.5	6.5	7.4	8.4
Ile	2.5	4.3	4.1	8.4
Leu	8.1	7.4	11.2	5.3
Phe	2.7	3.6	3.7	1.5
Met	1.4	1.6	0.9	1.5
Trp	0	2.1	0	0
	Carbohydrate residues per 100 amino acid residues			
	W3/13	L-C	Thy-1	Glycophorin A
Fucose	0	0.9	1.0	1.0
Mannose	0	4.4	10.6	4.0
Galactose	22.3	3.6	5.5	19.0
Glucose	11.1	1.1	1.3	0
Glucosamine	0	5.7	9.4	6.2
Galactosamine	20.1	1.1	0	11.1
Sialic acid	27.5	5.4	1.8	21.9
Sialic acid by fluorometric method	27.2	2.9	2.1	—
% by weight carbohydrate	60	25	32	56

The data for W3/13 and L-C antigens were determined as below; the data for rat thymocyte Thy-1 (L⁺ form) and human glycophorin are from refs 18 and 19, respectively. (The glycophorin carbohydrate composition was calculated from that given for the chymotryptic 1 peptide, which contains all the carbohydrate structures.) Amino acids were quantified on a Durrum D500 after 24, 48 and 72 h of hydrolysis in 6 M HCl and 0.005 M phenol *in vacuo* at 110 °C. Ser and Thr were calculated by extrapolation to zero time. Cys and Met were determined after performic acid oxidation⁴¹. The data for W3/13 and L-C were for five and four preparations, respectively. Trp in L-C antigen was detected spectrophotometrically⁴². No Trp was detected in W3/13 by the method described in ref. 43. The s.e.m.s were less than 5% of the mean value except for Met, Tyr and His, which were less than 12% of the mean value. Hexoses and amino sugars were determined by gas-liquid chromatography after methanolysis and trimethylsilylation⁴⁴. Sialic acid was also determined fluorometrically⁴⁵ with correction for interferences by hexoses and hexosamines. The carbohydrate analyses were from two to five separate preparations of W3/13 and L-C antigens. The s.e.m.s were less than 10% of the mean. The amino sugars were also analysed by ion exchange chromatography following hydrolysis in 3 M *p*-toluene sulphonic acid⁴³. The values (not shown) were similar to those obtained by gas-liquid chromatography. Glucose was also found in fractions from the Sephacryl S300 column which did not contain W3/13 antigen and was therefore assumed to be a contaminant. The percentage by weight of carbohydrate of W3/13 and L-C glycoproteins was determined using the sialic acid values obtained by the fluorescence method, assuming it was *N*-acetylneuraminic acid. Amino sugars were assumed to be *N*-acetylated.

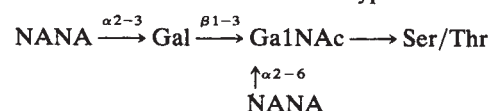
apparent MW, with other minor peaks at apparent MWs 86,000 and ~70,000. These are probably proteolytic products of W3/13 antigen as they stain heavily with PAS stain. This possibility was supported by an experiment in which the glycoprotein was run on SDS-PAGE followed by slicing the gel and assaying for W3/13 antigenic activity in material eluted from the gel. The results, which are considered to be semi-quantitative, are shown in Fig. 2c and antigenic activity is associated with the protein and carbohydrate bands shown in Fig. 2a and b. Small amounts of antigenic activity were also detected at an apparent MW of 58,000 where Coomassie blue staining bands cannot be seen. The overall conclusion from Fig. 2 is that the W3/13 antigen is sufficiently pure for determination of carbohydrate and amino acid compositions.

A final point regarding the purification is the relationship between the molecule which binds to the W3/13 antibody column and the whole of the 95,000 MW glycoprotein band. This glycoprotein appears homogeneous and the fact that all ³H-labelled material shows the unusual behaviour after neuraminidase digestion (see Fig. 1) would suggest that the whole band is structurally similar. Despite this, it is clear that the W3/13 monoclonal antibody binds only a part of the glycoprotein. When solubilized membrane is passed through the affinity column, although all W3/13 antigenic activity is bound (Table 1) a part of the 95,000 apparent MW or the asialo 105,000 apparent MW glycoprotein band does not bind (Fig. 1, track 4 compared with 3, and track 7 compared with 6). Radioactivity counting in gel slices indicates that about 50% of the glycoproteins did not bind. This is not an artefact of glycoprotein radiolabelling because the same result was seen if material passing through the antibody column was analysed by PAS staining after SDS-PAGE (data not shown). It seems that the monoclonal antibody detects antigenic heterogeneity in glycoprotein molecules with an overall similarity of structure, a view supported by the fact that mouse antibodies raised against the purified W3/13 antigen can react with the 95,000 apparent MW band which has passed unretarded through the W3/13 antibody affinity column (W.R.A.B., unpublished observations).

Chemical composition of the W3/13 antigen and the L-C antigen from thymocytes

The pure W3/13 and L-C antigens, purified as previously described, were analysed for their carbohydrate and amino acid contents. The results are given in Table 2 where they are compared with the compositions of rat thymocyte Thy-1 antigen and human erythrocyte glycophorin A.

The W3/13 antigen has a carbohydrate composition quite unlike either of the other two heavily glycosylated molecules of the rat thymocyte membrane. Carbohydrate comprises 60% w/w of the molecule and consists solely of galactose, galactosamine and sialic acid. (Some glucose was also detected but this is probably due to contamination; see Table 2 legend.) No mannose or glucosamine was detected and thus the molecule lacks carbohydrate structures of the type which are *N*-linked to asparagine through *N*-acetylglucosamine and have a core of glucosamine and mannose residues²¹. The carbohydrate composition of W3/13 glycoprotein is what would be expected if the molecule contained structures of the type



which are found attached to proteins by an *O*-glycosidic linkage to serine or threonine²¹. The absolute amount of galactosamine and galactose suggests that there are about 20 such structures per 100 amino acids and this is supported by the finding that Ser and Thr together constitute 27 mol % of the amino acids, an unusually high proportion.

The *O*-glycosidic linkage of carbohydrate to protein is alkali labile whereas the *N*-glycosidic bond is not. Alkali effects a

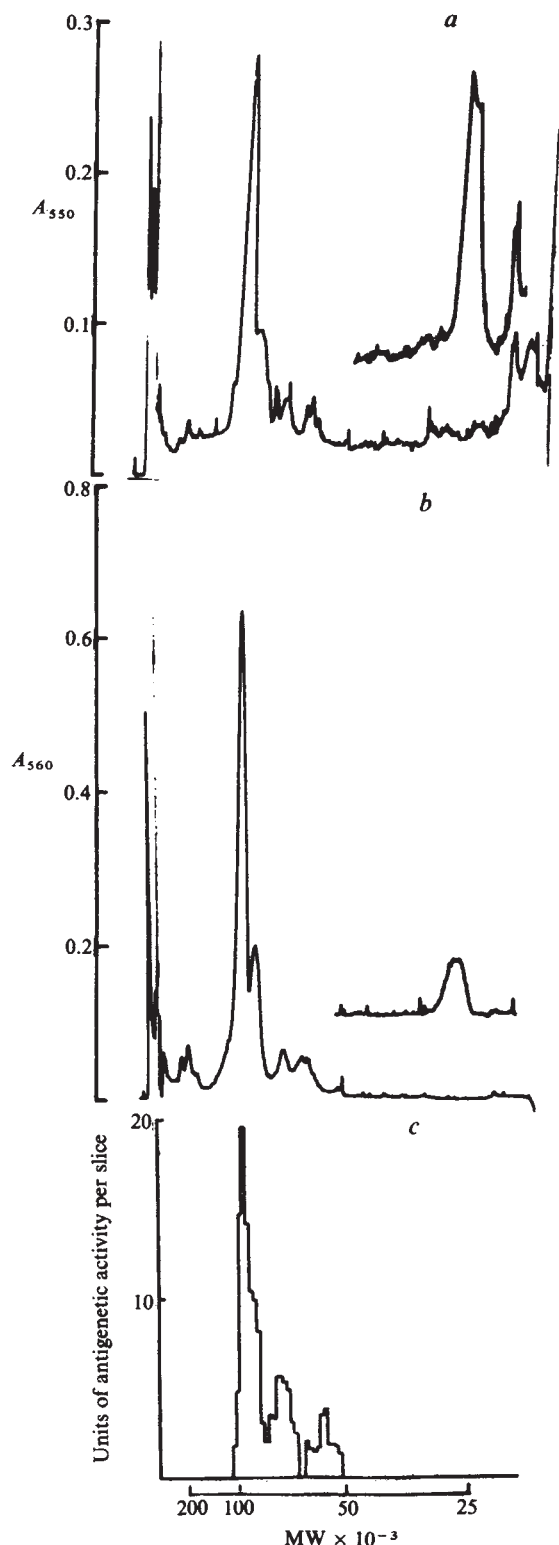


Fig. 2 SDS-PAGE of purified W3/13 antigen. W3/13 glycoprotein purified as in Table 1 was electrophoresed at 4 µg protein per sample on 11% polyacrylamide slab gels (3% stacking gel) and stained with Coomassie blue (a) and periodic acid Schiff stain (b) (methods as in ref. 6). The figure shows absorbance across the gel read by densitometry using a Gilford 2520 densitometer scanning at 550 nm for Coomassie blue and 560 nm for PAS stain. The insets show the traces from gels on which 4 µg protein of rat brain Thy-1 antigen was electrophoresed and stained with Coomassie blue (a) and PAS (b). c Shows the recovery of W3/13 antigenic activity after SDS-PAGE. A 3-µg protein sample of W3/13 glycoprotein was suspended in 0.1% SDS, 10% glycerol and electrophoresed as above. The gel was then sliced into 1-mm slices which were extracted overnight at 20 °C with 135 µl of 1% bovine serum albumin and 10 mM Tris-HCl, pH 8. The antigenic activity of the extracts was assayed by inhibition of an indirect binding assay for W3/13 antigen¹⁷. One unit of activity is the amount of antigen needed to give 50% inhibition of the standard assay. The total recovery of antigenic activity was 66% of that expected.

β -elimination of the carbohydrate attached to protein at serine or threonine residues. If this reaction is carried out in the presence of excess NaBH_4 , the aldehyde at the reducing terminus of the eliminated oligosaccharide is reduced to an alcohol²². Thus, if the carbohydrate of W3/13 antigen is *O*-linked to Ser and Thr, it should be released in a low MW form by β -elimination in the presence of excess NaBH_4 . This was examined using glycoprotein which had been labelled with tritium in its sialic acid or galactose residues (after removal of sialic acid). The results (Fig. 3) show that in both cases almost all the labelled carbohydrate was released by β -elimination into low MW forms which were retarded in gel filtration on a Bio-gel P-6 column. With asialo glycoprotein one low MW peak was obtained whereas alkali treatment of the sialylated glycoprotein gave two peaks. The latter two peaks presumably constitute structures containing one and two sialic residues. This is supported by the compositions (Table 2) which show that there is insufficient sialic acid for all structures to have two sialic acid residues as indicated in the structure above. The only unusual point about the results in Fig. 3 is that the ^3H -labelled oligosaccharides were retarded rather less than would be expected for their proposed MW in comparison with marker compounds chromatographed separately. The significance of this is unclear, but the data strongly suggest that all the sialic acid and galactose of the W3/13 antigen occurs in *O*-linked structures.

There is no similarity between the composition of the L-C antigen of rat thymocytes (glycoprotein MW 150,000) and W3/13 glycoprotein. Also, the amino acid content of L-C antigen shows no particular distinguishing features (Table 2); carbohydrate constitutes 25% w/w of the molecule, and the predominant saccharides present are mannose, galactose, *N*-acetylglucosamine and sialic acid. This suggests that the L-C antigen contains mainly *N*-linked carbohydrate, the low value for *N*-acetylgalactosamine arguing against a large number of *O*-linked structures. The compositions for L-C antigen are quite similar to those for Thy-1 antigen (Table 2). Brain Thy-1 antigen has three *N*-linked structures per polypeptide¹⁰ (111 amino acids) and thymocyte Thy-1 may have the same attachment points but with structures differing in the extent of completion at the terminal branches¹⁸. The L-C antigen has about half as many mannose and *N*-acetylglucosamine residues per 100 amino acids as Thy-1 antigen. This suggests that there are roughly 1.5 *N*-linked carbohydrate structures per 100 amino acids, and thus about 15 *N*-linked structures per molecule, assuming a molecular weight of 150,000. The amino acid composition of Thy-1 antigen and L-C antigen are similar, but this is uninformative as the molecules differ markedly in size and do not have unusual amino acid compositions.

Discussion

We have shown here that one of the three major glycoproteins of rat thymocytes is unusual in having an *O*-linked carbohydrate structure attached to about one amino acid in five. This must have a major effect on the structure of the molecule. Presumably there would be little tertiary folding of the polypeptide and the molecule probably adopts an extended conformation in solution^{23,24}. The expression of such a molecule at cell surfaces can be anticipated to have an important effect on the charge and topography of the outer surface. From the data in Tables 1 and 2 it can be calculated that on rat thymocytes 4×10^6 molecules of sialic acid are attached to the W3/13 antigen and probably twice this amount if all of the 95,000 MW glycoprotein is taken into account. This compares with $\sim 2 \times 10^6$ molecules of sialic acid per cell on Thy-1 antigen (two residues per molecule, 10^6 molecules per cell) and the same number on L-C antigen (30 residues per molecule and 70,000 molecules per cell). On both T lymphocytes and bone marrow neutrophils there is at least as much W3/13 antigen as on thymocytes¹⁷. An equivalent glycoprotein molecule can be identified on the cell surface of mouse thymocytes and T lymphocytes^{3,4} and it seems possible that the major glycoprotein of 90,000 MW which has been identified on mouse neutrophils²⁵ is the equivalent of the rat W3/13 antigen.

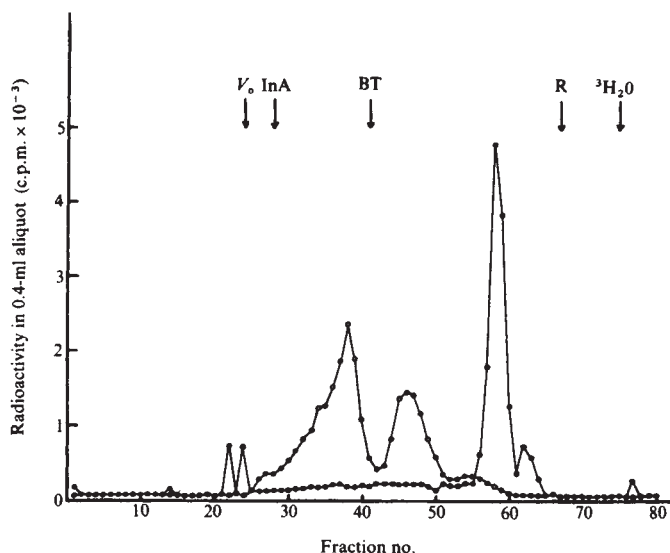


Fig. 3 Gel filtration of oligosaccharides derived by alkaline-borohydride degradation of ^3H -W3/13 antigen. ^3H -labelled W3/13 was prepared as described in Fig. 2 legend from thymocytes labelled with NaB^3H_4 after mild periodate oxidation ($\circ$) or after incubation with neuraminidase and galactose oxidase ($\bullet$). The ^3H -labelled W3/13 was dialysed into distilled water and then subjected to alkaline borohydride degradation as described in ref. 22. After destruction of the excess NaBH_4 with acetic acid the mixture was fractionated on a Bio-gel P-6 column ($102 \times 1 \text{ cm}$) in $0.1 \text{ M NH}_4\text{HCO}_3$ at a flow rate of 4 ml h^{-1} . Fractions (1.1 ml) were collected of which 0.4-ml aliquots were scintillation counted in 3 ml Biofluor (NEN). The positions of elution of the following markers are shown. InA, insulin A, MW 2,360; BT, bacitracin, MW 1,460; R, raffinose, MW 504.

The expression of W3/13 antigen on T but not B lymphocytes may contribute to some of the differences between these cell types. Rodent T lymphocytes can be separated from B lymphocytes by electrophoresis because T cells are more acidic²⁶. The presence of W3/13 antigen on T lymphocytes may be the reason for this difference in charge. Also, T lymphocytes are generally regarded as being less 'sticky' than B cells²⁷ and *in vivo* T cells recirculate more rapidly from blood to lymph²⁸. Such differences could be mediated by an extended cell-surface molecule with a high concentration of negative charges which acts to impede surface contact.

Molecules with a high content of O-linked carbohydrate have been described from a variety of sources. A serum glycoprotein of this type is the anti-freeze protein of fishes which live at sub-zero temperatures²⁹. A glycoprotein with similarities to the W3/13 antigen has also recently been purified from human serum³⁰. Whether it might have originally derived from cell surfaces is not known. This molecule has an apparent MW of 140,000 on SDS-PAGE and contains 76% w/w carbohydrate, most of which is O-linked. Studies on this molecule suggest that it has a very extended conformation and little secondary structure³⁰. Membrane or glycocalyx molecules with a high content of O-linked carbohydrates have also been described²¹. These include mucins which form a glycocalyx at epithelial cell surfaces and glycoproteins isolated from ascites tumour cells. These molecules seem to be much larger than the thymocyte glycoprotein described here and in the case of the tumour cell glycoproteins it has been suggested that the molecules mask other cell-surface structures^{31,32}.

The membrane molecule most like the W3/13 antigen is glycophorin, the major sialoglycoprotein of human erythrocytes. This molecule has 15 O-linked carbohydrate structures plus one N-linked structure attached to a sequence of 131 amino acids¹⁹. The amino acid and carbohydrate compositions for glycophorin are given in Table 2 and the similarities between this molecule and W3/13 glycoprotein are striking. Both molecules lack Cys and Trp and have low amounts of basic residues and high values for Ser, Thr and Pro. Significant differences are seen in the amounts of His, Ala, Gly, Tyr, Ile and Phe. In the

carbohydrate compositions the N-linked structure of glycophorin is indicated by the presence of mannose and glucosamine. The similarities between the W3/13 glycoprotein and glycophorin suggest that these molecules may be evolutionarily related (alternatively, the similarities in amino acid composition could simply be a consequence of the unusual structure). There is more than one gene coding for the human erythrocyte glycophorins^{33,34} and a family of genes, some members of which are expressed on other cells, could have evolved by gene duplication. The W3/13 antigen is not found on rat erythrocytes¹⁷ but these cells are likely to express other glycophorin-like molecules³⁵.

The function of glycophorin is unknown and its importance has been questioned following identification of individuals who lack the molecule but show no defect in erythrocyte function^{36,37}. This finding is difficult to evaluate because the expression of carbohydrate on other erythrocyte membrane molecules is altered in these individuals³⁶. The finding of a glycophorin-like molecule as a major constituent of a number of other haematopoietic cell types (and possibly brain) strongly suggests that molecules of this type are functionally important. Their main role may be to alter the charge characteristics of the cell surface, particularly at a distance from the phospholipid bilayer. The expression of such molecules may affect the ease with which the membrane can align with that of another cell and thus be an important physical constraint in cell interactions.

We thank Mr W. Potts for assistance with carbohydrate analyses, Mr T. Gascoyne of the MRC Immunochemistry Unit, Oxford, for amino acid analysis and Mrs J. Kosak for typing the manuscript. W.R.A.B. was supported by an SRC research studentship.

Received 28 August; accepted 5 November 1980.

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The reactivation of developmentally inert 5S genes in somatic nuclei injected into *Xenopus* oocytes

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In the frog Xenopus, the somatic-type 5S ribosomal RNA genes are active in all cells; the oocyte-type 5S genes are active in oocytes but not in somatic cells. A new method of native gel electrophoresis resolves the two types of 5S RNA which are of the same length but different sequence. When somatic nuclei were injected into oocytes, their inactive oocyte-type 5S genes often remained inactive, and had thus conserved their developmentally regulated condition. Other conditions, which include treatment with 0.35 M NaCl, reactivated the genes.

THERE are two classes of 5S ribosomal RNA genes in the genome of *Xenopus* and other amphibians. One class, comprising the oocyte-type 5S genes, is expressed in oocytes, but not in somatic cells^{1,2}. The other class, the somatic-type 5S genes, is expressed in both oocytes and somatic cells². We are investigating the mechanism by which the oocyte-type 5S genes are inactivated in somatic cells, as this may elucidate the mechanism by which the cell type-specific expression of genes is achieved in development.

The transcription of cloned 5S genes has been studied extensively with purified DNA in injected oocytes³⁻⁵ as well as in cell-free systems⁶⁻¹³. However, in none of these cases is the natural pattern of differential transcription between oocyte- and somatic-type genes reproduced; in injected oocytes and somatic cell extracts, both types of 5S gene are transcribed. As we believe that genes in an intact nucleus are more likely to exhibit regulated transcription, we have injected whole somatic nuclei into oocytes. Then, if the oocyte-type 5S genes are activated, we can investigate changes associated with activation; but, if these genes remain inactive after injection, we can determine which components of the nuclei must be removed to obtain reactivation.

Previous work encourages this approach. We found that somatic nuclei injected into *Xenopus* oocytes are transcriptionally active for many days¹⁴. In one series of experiments, a gene product made in HeLa cells continued to be synthesized by HeLa nuclei injected into oocytes¹⁵. In other experiments, genes characteristically inactive in somatic cells were activated after the injection of nuclei into oocytes¹⁶.

We describe here different conditions in which the oocyte-type 5S genes in somatic nuclei can either be made to remain inactive or can be reactivated after their injection into oocytes. To assay for selective transcription of oocyte- or somatic-type 5S RNA, we have developed a generally useful procedure for distinguishing between different RNAs of identical length. Although we do not yet know what factors are responsible for the developmental regulation of 5S gene transcription, this new approach to studying the expression of specific genes as part of an intact nucleus can be used to yield information on the control of these genes.

Rapid resolution of different RNAs of the same length

The oocyte and somatic types of *Xenopus* 5S RNAs are the same length (120 nucleotides) and differ from each other in sequence by six nucleotides in both *Xenopus laevis* and *Xenopus borealis*^{17,18}. All four RNAs co-migrate on a denaturing polyacrylamide gel (Fig. 1a). The four types can be distinguished by RNA fingerprinting¹⁷, but this procedure is too laborious for use with numerous samples. We have developed a simple electrophoresis assay which distinguishes all four 5S RNAs, and

which is suitable for the analysis of many samples simultaneously.

X. laevis and *X. borealis* oocyte- and somatic-type 5S RNAs are unambiguously resolved by electrophoresis through a non-denaturing 17% polyacrylamide gel prepared and run as described in Fig. 1 legend. The substantially different mobilities of these four RNAs probably reflect differences in conformation in these conditions. In modified conditions which favour less secondary structure (elevated temperature, urea), resolution is considerably decreased. For our purposes, the main value of this method is for the rapid determination of the relative synthesis of oocyte- and somatic-types of 5S RNA (Fig. 1b).

The resolving power of this gel system, however, is not limited to 5S RNAs. The yeast tyrosine tRNA SUP4 and a mutant tRNA U67 are both 78 nucleotides long, and differ only by a single base change at position 67 (C→U)¹⁹. These tRNAs migrate with different mobilities on native gels (Fig. 1c), though they migrate together on denaturing gels²⁰. Similar results have been obtained for other tRNAs.

5S genes are efficiently transcribed in injected nuclei

Cultured cell nuclei injected into living oocytes are transcriptionally active. Oocytes injected with as few as 100–200 nuclei synthesize many times more somatic-type 5S RNA than uninjected control oocytes (Fig. 2, lanes 1–9 compared with lanes

Table 1 Activation of oocyte-type 5S genes in erythrocyte nuclei injected into oocytes

	Oocytes injected with erythrocyte nuclei	Adult somatic cells		
		Liver	Tissue culture cells	Factor of activation
Oocyte-type 5S RNA	40	<0.05	<0.07	800
Somatic-type 5S RNA	1	1	1	

Erythrocyte nuclei prepared by lysolecithin treatment were injected into oocytes, aiming for the germinal vesicle. Four hours later 1 μ Ci [α -³²P]GTP was injected, and the oocytes labelled for 2 days. Subsequent analysis was as for Figs 1, 2. The relative intensities of oocyte- and somatic-type 5S RNA bands were measured by microdensitometry. Data presented are the average of seven samples from two experiments. To determine the relative synthesis of oocyte- and somatic-type 5S RNA in somatic cells, an adult *X. laevis* was injected with 5 mCi ³²P₀₄. After 4 days, the liver was removed and the RNA extracted¹⁶. The sample was electrophoresed with a 5S RNA marker on a 12% polyacrylamide 7 M urea gel, the 5S RNA region excised and the RNA eluted from the gel¹⁷. The RNA was then re-electrophoresed on a 17% polyacrylamide native gel as described in Fig. 1 legend. *Xenopus laevis* kidney tissue culture cells were labelled for 24 h and the RNA isolated and electrophoresed as described in Fig. 3 legend. The relative intensities of oocyte- and somatic-type 5S RNA bands were determined. The absence of oocyte-type 5S RNA in tissue culture cells may be due to rapid turnover of such transcripts. However, we also failed to detect any oocyte-type 5S RNA when cultured cells were labelled for only 4 h.

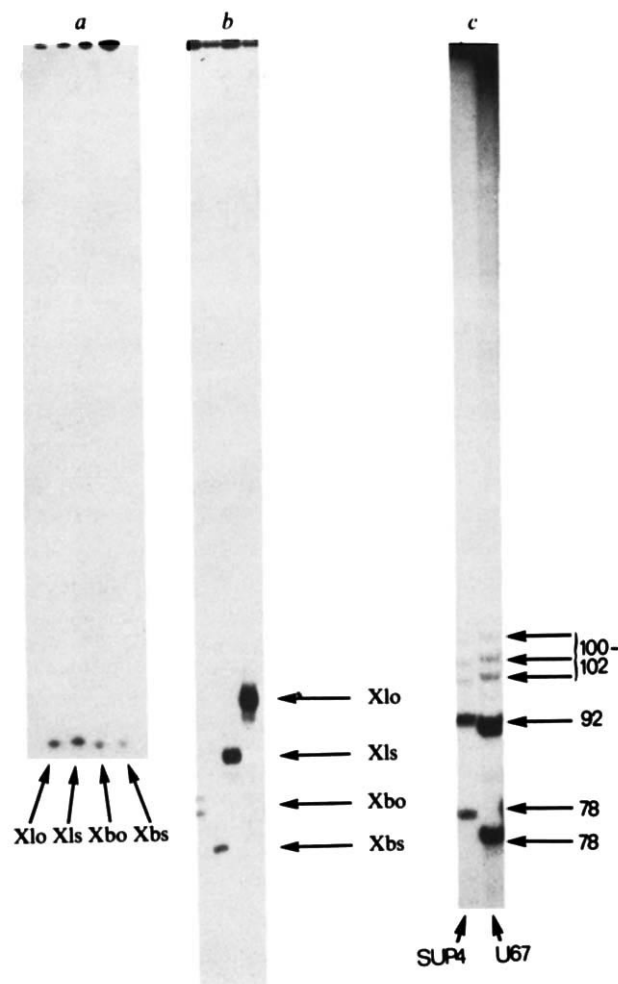


Fig. 1 Separation of RNA molecules by gel electrophoresis in non-denaturing conditions. *a*, Autoradiogram of a denaturing 12% polyacrylamide-7 M urea gel³⁸ of ³²P-RNA synthesized in *Xenopus* oocytes when [α -³²P]GTP and pXlo 8, pXls 11, pXbo 3, or pXbs 1 5S DNA were injected. pXlo 8 contains four *X. laevis* oocyte-type 5S genes⁴. pXbo 3 contains six *X. borealis* oocyte-type 5S genes^{4,39}. pXls 11 and pXbs 1 contain one repeating unit each of *X. laevis* and *X. borealis* somatic-type 5S DNA, respectively²³. About 5 ng DNA and 1 μ Ci [α -³²P]GTP were injected into the germinal vesicle of each oocyte. The oocytes were incubated at 20 °C for 24 h and the RNA extracted by the method of Brown and Litt⁴⁰. The gel is 0.35 mm thick and contains 12% polyacrylamide, 7 M urea, 0.05 M Tris borate, pH 8.3, and 1 mM EDTA. For all gels, hypersensitized film was exposed at -70 °C using a Dupont lightning plus intensifying screen. RNA transcripts from the cloned DNAs, following injection into oocytes, have been sequenced and identified as oocyte- or somatic-type 5S RNA from the respective *Xenopus* species^{4,18,23}. *b*, Autoradiogram of a native 17% polyacrylamide gel of ³²P-RNA synthesized in *Xenopus* oocytes when [α -³²P]GTP and pXlo 8, pXls 11, pXbo 3, or pXbs 1 5S DNA were injected. DNA injections, and conditions for the synthesis and extraction of 5S RNA are as described for *a*. After ethanol precipitation and two 70% ethanol washes, the RNA sample was redissolved in 1–5 μ l 10% glycerol, 1 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue. The 17% polyacrylamide (1:20 bisacrylamide/acrylamide), 0.35 mm-thick gel was prepared in 40 mM Tris, 20 mM Na acetate and 2 mM EDTA, to pH 7.8 with acetic acid. The electrode buffer was 50 mM Tris, 0.4 M glycine and 1 mM EDTA, pH 8.3. Electrophoresis was at constant current (9–11 mA) for 24 h. 5S RNA migrates through the gel at a rate of approximately 1 cm kV⁻¹ h⁻¹. For maximum resolution, the gel should not be allowed to exceed 30 °C. The pXbo 3 clone contains two types of Xbo gene (ref. 39, and L. J. K. and D. D. Brown, unpublished). The 5S RNA transcripts from these genes separate on the native gel. *c*, Autoradiogram of a native 17% polyacrylamide gel of ³²P-RNA synthesized in *Xenopus* oocytes when [α -³²P]GTP and pSUP4 or pU67 DNA were injected. pSUP4 contains the yeast Tyr tRNA SUP4 gene⁴¹ and pU67 contains a single base change (C $\rightarrow$ U) at position 67 of the mature tRNA⁴². *Xenopus* oocytes transcribe and process these yeast tRNA genes⁴². The mature tRNAs are 78 nucleotides long. DNA injections, conditions for the synthesis and extraction of RNA and gel electrophoresis were as described above. tRNA precursor molecules of 92 and 100–102 nucleotides are indicated. Note that although the U67 mature tRNA migrates faster than SUP4, the 92- and 100–102-nucleotide precursor molecules do not. Therefore, the separation of the two 78-nucleotide molecules cannot be explained by the samples in one lane of the gel migrating faster than those in another.

10–12). The transcription of 5S genes in injected nuclei continues for at least 4 days (Fig. 2, lanes 7–9), and would probably continue for as long as the oocytes themselves remain synthetically active (sometimes for a few weeks). The rate of somatic-type 5S RNA synthesis has been estimated from the specific activity of the ³²P-GTP pool of oocytes (2.5 Ci mmol⁻¹), and from the number of ribosomes ($\sim 10^7$) synthesized per cell cycle in cultured cells. From these calculations, the rate of somatic-type 5S RNA synthesis in somatic nuclei injected into oocytes (500 transcripts per gene per h) seems to be at least as much as in the same nuclei when they were present in somatic cells (350 transcripts per gene per h). Thus this efficient transcription produces sufficient material from a single oocyte injected with nuclei to determine whether the 5S RNA synthesized is the oocyte or somatic type. As 5S transcription by injected nuclei is so much more than the endogenous level, the following results reflect the activity of 5S genes in the injected somatic nuclei and not that of the oocytes' own 5S genes.

The efficient transcription of nuclei injected into oocytes can be compared with that of isolated somatic nuclei incubated *in vitro*. Using two procedures optimized for transcription by RNA polymerase III (refs 6, 21), we found that 10⁷ nuclei from *Xenopus* cultured cells incubated for 2 h synthesized only just enough 5S RNA to be detected (Fig. 2, TCN), or 0.025 transcripts per gene per h.

Activation or non-activation of oocyte-type 5S genes

Before assessing changes in 5S gene activity, we must establish the level of their activity before injection—in normal somatic cells and in oocytes. In somatic cells (see Table 1; Fig. 3, TCC; ref. 22) there are at least 20-fold more somatic-type 5S transcripts than oocyte-type 5S transcripts. This contrasts with the 5S gene copy number which in *X. laevis* favours the oocyte-type 5S genes by 50:1 (ref. 23). Thus, each somatic-type 5S gene is approximately 1,000 times more active in somatic cells than each oocyte-type gene. In the oocyte, on the other hand, the oocyte-type 5S transcripts predominate (oocyte/somatic 5S RNA ratio approximately, 20:1) (ref. 2 and unpublished results). Note that, unlike the 18S and 28S ribosomal RNA genes, gene amplification is not responsible for this preferential expression of oocyte-type 5S genes in oocytes^{24,25}, the same number of these genes (20,000 per haploid chromosome set in *X. laevis*) being present in oocytes and in somatic cells.

As shown in Fig. 2, oocyte-type 5S genes of injected somatic nuclei can remain inactive after introduction into oocytes. Furthermore, this inactivity persists unaltered for at least 4 days. However, in several experiments we have observed the opposite result—an activation of oocyte-type 5S genes. Figure 3 shows the result of injecting samples of the same nuclear suspension into oocytes of two females. In the oocytes of one female,

Table 2 NaCl activation of oocyte-type 5S genes

Ratio of oocyte 5S/somatic 5S	No. of individual assays			
	Lysolecithin		Triton	
	-NaCl	+NaCl	-NaCl	+NaCl
>1 (activation)	14	7	1	14
<1 (no activation)	122	2	5	3
No. activated/no. not activated	0.11	3.5	0.2	4.7
Effect of NaCl ($\frac{\text{ratio} + \text{NaCl}}{\text{ratio} - \text{NaCl}}$)	31x		24x	

An activated oocyte is one in which, following injection of somatic nuclei, the amount of oocyte-type 5S transcripts is greater than somatic-type 5S. No activation means somatic-type 5S transcripts exceed oocyte-type ones. Nuclei were prepared from *X. laevis* tissue culture cells by lysolecithin or Triton treatment²⁶. Salt-washed nuclei were incubated for 15 min at 0 °C in 0.35 M NaCl at a concentration of 5×10^6 nuclei ml⁻¹ before injection. Approximately 500 nuclei were injected into single oocytes from non-activating females, aiming for the germinal vesicle. Each assay is a single oocyte.

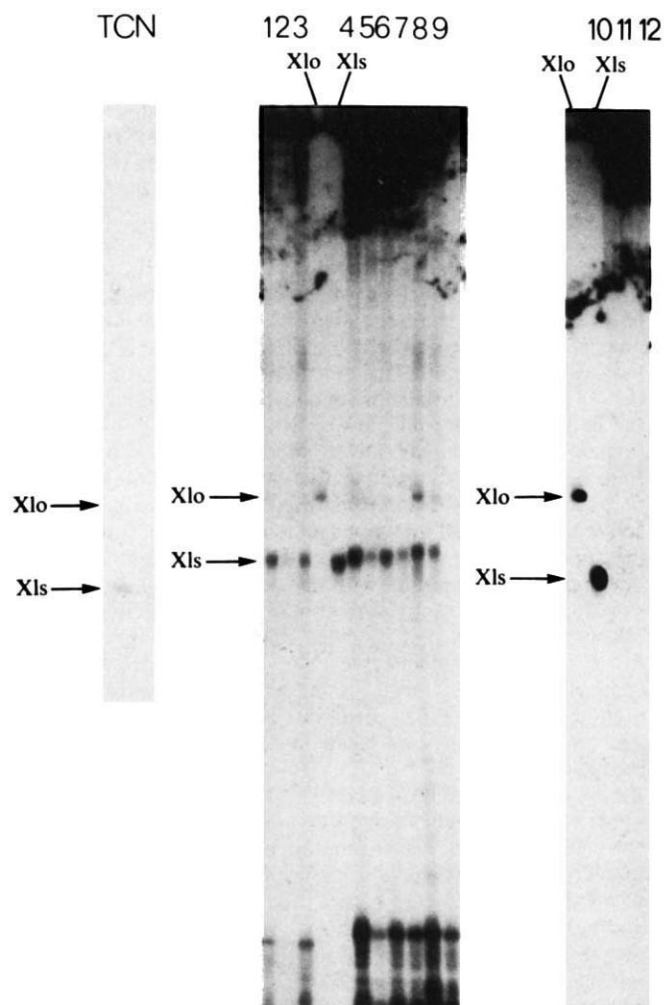


Fig. 2 5S gene transcription by somatic nuclei injected into oocytes. Tissue culture nuclei (TCN) were prepared by Triton treatment²⁶ from a line of *X. laevis* kidney cells growing in culture. 10^7 nuclei and $50 \mu\text{Ci}$ [α - ^{32}P]GTP were incubated for 2 h at 25°C as described by Crampton and Woodland²¹. The RNA was extracted and electrophoresed on a 12% polyacrylamide-7 M urea gel and the 5S RNA band was located by its co-migration with Xlo and Xls markers (see Fig. 1a). The 5S RNA band was excised and the RNA eluted³⁷. The RNA was then electrophoresed on a 17% polyacrylamide native gel (track TCN). The positions of *X. laevis* oocyte- and somatic-type 5S RNAs which have been eluted from the denaturing gel and then electrophoresed on the native gel are indicated with arrows. Lanes 1–12, between 100 and 500 tissue culture cell nuclei prepared by lysolecithin treatment²⁶ were injected into individual oocytes, aiming for the germinal vesicle, followed by a second injection of $1 \mu\text{Ci}$ [α - ^{32}P]GTP at 4 h (lanes 1–3), 24 h (lanes 4–6) or 4 days (lanes 7–9) after the first injection. Tissue culture cells were removed from culture dishes by scraping rather than with trypsin because nuclei prepared from trypsin-treated cells were often toxic to oocytes. Control oocytes were injected with [α - ^{32}P]GTP only (lanes 10–12). The endogenous oocyte transcription contributes to the high-molecular-weight RNA at the top of the gel. In this and all subsequent figures, the RNA was analysed as in Fig. 1 legend and half the RNA from individual oocytes electrophoresed in each lane of the native gel. tRNA molecules (bottom of gel) provide an internal control for the relative efficiency of labelling of RNA transcripts in different oocytes. Occasionally, a modest activation of oocyte-type 5S genes is observed (for example, lane 8); note, however, that Xlo 5S genes outnumber Xls genes by 50:1 (ref. 23).

Table 3 5S DNA-binding protein does not activate oocyte-type 5S genes

	Concentration ($\mu\text{g ml}^{-1}$)	No. of oocytes with oocyte-type 5S genes		
		Fully activated	Partially activated	Not activated
7S particle	1.5	0	0	7
7S particle	7.5	0	0	8
7S particle	60	0	2	5
Protein	0.8	0	0	8
Protein	3.8	0	0	8
Protein	30	0	0	8
None	—	0	1	7

Erythrocyte nuclei (about 200 per oocyte) were mixed with 7S particle or purified protein and injected into non-activating oocytes. The concentrations given are those of the material as injected. If the injected material was evenly dispersed throughout the oocyte, the intracellular concentration would have been about 10 times less (50 nl injected into $1 \mu\text{l}$ oocytes, of which about half the volume is solid yolk). The concentration of particle and protein used by Pelham and Brown³³ and in our experiments (purified as described in refs 32, 33) to give two- to six-fold stimulation of 5S DNA transcription ranged from <1 to $>20 \mu\text{g ml}^{-1}$. Injected oocytes are classified as partially activated when the ratio of labelled oocyte-type to labelled somatic-type 5S RNA was between 1:1 and 3:1, and as not activated when this ratio was 1:1 or less. Note that we never observed full activation (ratio $\geq 10:1$). In a second experiment, in which again no evidence of activation was obtained by 7S particle or protein, full activation (oocyte-type/somatic-type 5S RNA ratio of $>10:1$) was observed when nuclei were treated with 0.35 M NaCl and Triton (as in Table 2 legend).

type/somatic-type 5S RNA ratio of <1 . Seven females had oocytes which gave intermediate values.

Note two aspects of these results. First, oocytes taken from the same female at intervals over a few months always (six females tested 2–4 times each) show the same activating or non-activating qualities as initially determined. Second, the pattern of somatic-type 5S transcription and tRNA synthesis in injected oocytes is approximately the same whether or not the oocyte-type 5S genes are activated (see, for example, Fig. 3). Somatic-type 5S RNA therefore provides an essential control, eliminating the possibility that the inactivity of oocyte-type 5S genes could be due to inviability of the injected nuclei in these oocytes. We conclude that the developmental inactivation of oocyte-type 5S genes takes place by a process that is readily reversed by components of the oocytes of some but not all females.

We have considered whether the oocyte-type 5S transcripts observed after the injection of somatic cell nuclei into activating oocytes could have resulted from a stimulation of transcription of the oocytes' endogenous 5S genes. This possibility has been eliminated by injecting *X. laevis* nuclei into oocytes from an activating *X. borealis* female. If the oocyte-type transcripts were coded for by the injected nuclei, they would be Xlo-type 5S RNA; however, if they were transcribed from the oocytes' own genes, they would be the Xbo type. The 5S RNA obtained co-migrates with Xlo, and not with Xbo (Fig. 3, lanes 9–11). Thus we conclude that the injected somatic nuclei are indeed activated to synthesize oocyte-type 5S RNA, and that the factor(s) responsible for this activation is not species specific.

5S gene activation in mature erythrocytes

Activation of 5S oocyte-type genes from cultured cell nuclei injected into oocytes might be due to the cell line becoming aberrant during many years of culture, as reported for a particular cultured line of *Xenopus* cells in which up to 15% of 5S transcripts are of the oocyte type²². We have therefore repeated our experiments with the nuclei of adult *Xenopus* erythrocytes to assess whether the inactivation of genes in a highly specialized, non-dividing, terminal cell type is also readily reversible.

Nuclei prepared by lysolecithin treatment²⁶ of mature erythrocytes of an adult frog were injected into oocytes of activating and non-activating females. Consistent with our results using nuclei from tissue culture cells, we find that erythrocyte nuclei injected into oocytes from non-activating females express somatic-, but not oocyte-type, 5S genes. In oocytes from activating females, on the other hand, the synthesis of oocyte-type 5S RNA was 40 times that of somatic 5S RNA (Table 1),

oocyte-type 5S genes were activated (Fig. 3, lanes 1–4); in the oocytes of the other they remained quiescent (lanes 5–8). Altogether we have tested oocytes from 50 females. Of these, 27 were activating, such that the amount of oocyte-type 5S RNA was between 5- and 40-fold greater than that of somatic-type 5S RNA. Sixteen females were non-activating, with an oocyte-

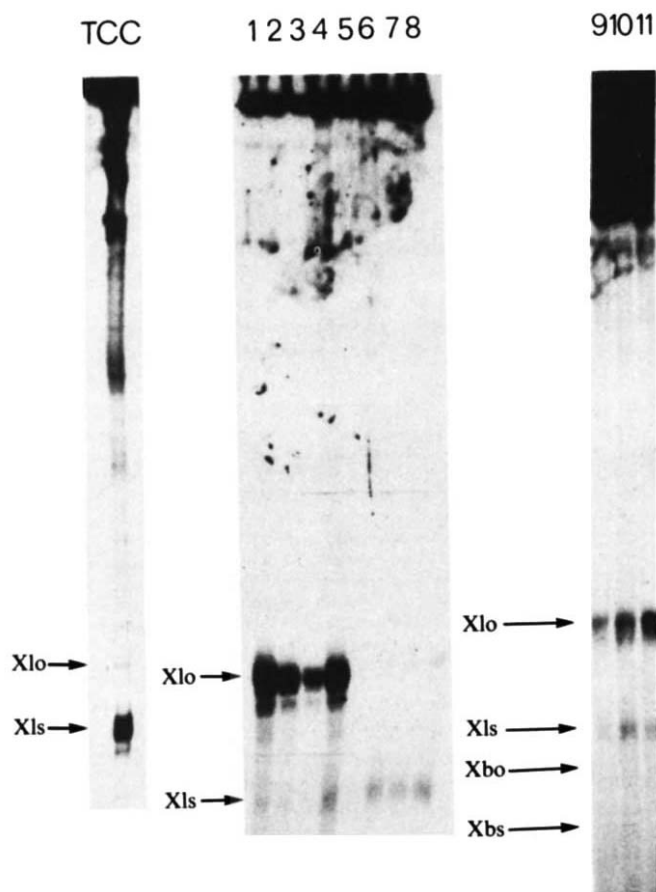


Fig. 3 Activating and non-activating oocytes. TCC, a 50% confluent flask of *X. laevis* kidney tissue culture cells (TCC) was incubated with 2 mCi ^{32}P inorganic phosphate for 24 h. Washed cells were lysed in isotonic buffer essentially as described by Kumar and Lindberg⁴³, except that the Nonidet P40 concentration was reduced to 0.2%. The RNA was electrophoresed on a 17% polyacrylamide native gel as described in Fig. 1 legend. Lanes 1–8, about 500 tissue culture cell nuclei prepared by lysolecithin treatment were injected with 1 μCi [α - ^{32}P]GTP into individual oocytes from *X. laevis* female A (lanes 1–4) or female B (lanes 5–8), aiming for the germinal vesicle. Labelling was for 24 h. Lanes 9–11, approximately 300 *X. laevis* kidney TCC nuclei prepared by lysolecithin treatment were injected into individual *X. borealis* oocytes, aiming for the germinal vesicle, followed by a second injection of 1 μCi [α - ^{32}P]GTP 4 h later. Labelling was for 40 h. The positions of *X. laevis* and *X. borealis* oocyte- and somatic-type 5S RNA, prepared as described in Fig. 1 legend, are indicated by arrows. tRNA synthesis, as seen at the bottom of the gel but not shown, was unaffected by the activated or non-activated state of the oocyte-type 5S genes.

just as was found in fully activated cultured cell nuclei. Similar results were obtained with a preparation of nuclei from immature blood cells taken from an adult *Xenopus* which had been rendered anaemic by the injection of phenylhydrazine 14 days earlier²⁷. The 40-fold excess of oocyte over somatic 5S RNA represents a major activation of oocyte-type 5S genes, comparable with the endogenous ratio in oocytes (20–40:1). In somatic cells, as described above, the transcription of oocyte-type 5S RNA is typically less than 5% of that of the synthesis of somatic 5S RNA. We confirmed this by measuring the ratio of oocyte to somatic 5S RNA synthesis in liver, which is the erythropoietic organ of *Xenopus* adults (Table 1). Thus erythrocyte nuclei undergo at least an 800-fold activation of their oocyte-type 5S genes after injection into oocytes (see Table 1 legend for comments on this calculation). We conclude that the developmental inactivation of 5S genes is as easily reversed in a highly specialized, normal, adult cell type as it is in a cultured line of cells.

Oocyte components required for gene activation

The germinal vesicle is the site of 5S gene activation. To help identify the components responsible for inactivating oocyte-type 5S genes in somatic cells, we have investigated the intracellular location and general nature of the activity concerned. If the activity is localized in the oocyte's nucleus or germinal vesicle (GV), gene activation should occur only when nuclei are injected into the GV and not when injected into the cytoplasm. The site of injected nuclei has been determined by examination of stained oocyte sections, as well as by the co-injection of purified DNA known to be transcribed only in the GV^{3,28}. In all the experiments reported above, injected nuclei were aimed at the oocyte's GV, and sections of oocytes revealed that in 90% of the cases, nuclei are indeed deposited there (Fig. 4). The co-injection of *X. borealis* DNA (Xbs) and whole nuclei was followed by analysis using native gel electrophoresis to distinguish Xb and Xl transcripts (Fig. 1b); Xbs DNA was transcribed in nearly all injections aimed at the GV, but never in injections aimed at the cytoplasm. The same type of analysis of nuclei injected together with DNA into activating oocytes established that the activation of oocyte-type 5S genes does not take place in the recipient oocytes' cytoplasm, though it did so in the GV of the same oocytes. Somatic-type 5S genes were transcribed by nuclei located in the cytoplasm as well as by those in the GV, although the overall rate of somatic 5S transcription was about 10 times less when nuclei were deposited in the cytoplasm.

Treatment with NaCl activates oocyte-type 5S genes. Another approach to the analysis of 5S gene activation by oocytes is to ask whether the removal of components of somatic nuclei before injection facilitates the effect. We have treated suspensions of nuclei in various ways before injecting them into oocytes of non-activating females, and have then assessed the extent of oocyte-type 5S activation by native gel electrophoresis. The results, which are summarized in Table 2, confirm that nuclei prepared by lysolecithin or Triton treatment alone only rarely synthesize more oocyte- than somatic-type 5S RNA. In contrast, 15 min incubation in 0.35 M NaCl at 0 °C, a procedure designed to remove most non-histone proteins²⁹, usually results in a pronounced activation of oocyte-type 5S genes (oocyte/somatic 5S ratio 10–30:1). In one experiment where nuclei were treated with 2 M NaCl and 0.5% Triton (Cook nucleoids)³⁰, a procedure which depletes almost all nuclear proteins, oocyte-type 5S genes were fully activated to give an oocyte/somatic 5S RNA ratio of over 30:1. Note that the 0.35 M NaCl treatment can activate the oocyte-type 5S genes to the same extent as in Cook nucleoids. Although the effect of removing materials from nuclei before injection is substantial, we have had occasional experiments in which NaCl treatment has little if any effect. We have not yet distinguished several possible explanations for the effect of NaCl.

The 5S DNA-binding protein does not cause activation. Engelke *et al.*³¹ have isolated a protein from *Xenopus* ovaries that binds to the part of the coding region of 5S genes known as the control region^{12,13}, and which is required specifically for the transcription of both oocyte- and somatic-type 5S DNA *in vitro*. Recently, it has been shown that this same protein combines with 5S RNA to form a 7S particle characteristic of *Xenopus* ovaries^{32,33}. These observations raise the possibility that this 5S DNA-binding protein may be responsible for 5S gene activation in oocytes.

We have tested this possibility by injecting oocytes of a non-activating female with somatic nuclei as well as with 5S DNA-binding protein or the 7S particle which contains this protein. We found no effect of either the protein or 7S particle on the reactivation of oocyte-type 5S genes in injected somatic nuclei (Table 3) or on total 5S transcription. We have tested the protein and particle on oocytes of two different non-activating females, and over several concentrations ranging from 1- to 25-fold the concentration required to give maximum stimula-

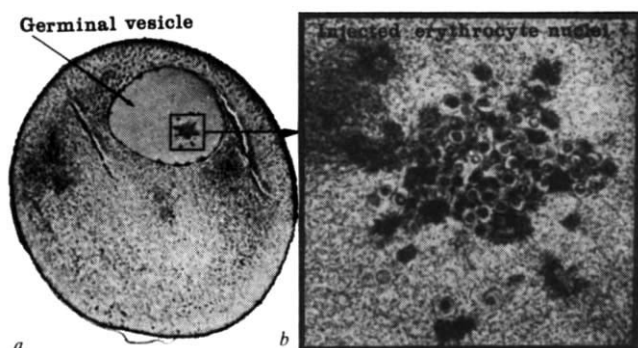


Fig. 4 Erythrocyte nuclei in injected oocytes. Oocytes were injected with a few hundred adult *Xenopus* erythrocyte nuclei prepared by the lysolecithin procedure²⁶, fixed after 1 day²⁶, sectioned and stained. **a**, The position of injected nuclei in the oocyte nucleus (germinal vesicle). **b** Shows an enlargement of the injected nuclei. Some of the nuclei appear to have formed dense-staining masses of chromatin, similar to those sometimes observed when HeLa nuclei are deposited in an oocyte germinal vesicle (see Fig. 3b of ref. 26). We do not know whether the 5S gene transcription which we observe comes from the chromatin masses or intact nuclei, both of which are seen in this oocyte section.

tion *in vitro*³³. Pelham and Brown³³ showed that both protein and 7S particle independently stimulate transcription of 5S DNA *in vitro* by a factor of 2–6. As the preparations of protein and particle which we used also stimulated 5S DNA transcription when tested *in vitro*, we conclude that these substances are not sufficient for the reactivation of oocyte-type 5S genes in somatic nuclei.

Discussion

The rapid reactivation of the developmentally inert oocyte-type 5S genes in somatic nuclei, following their injection into oocytes, demonstrates the ready reversibility of gene expression in development, and is consistent with previous work involving the introduction of nuclei into *Xenopus* eggs or oocytes. The development of tadpoles from transplanted adult skin cell nuclei implies the reactivation of developmentally inert genes³⁴. However, in this case, many cell divisions take place before reactivation is observed. More recently, two-dimensional gel analysis of proteins was used to demonstrate the synthesis of new proteins which are not expressed in a cultured line of *Xenopus* kidney cells, when nuclei from these cells were injected into oocytes¹⁶. The reactivation of gene expression is particularly clear in the case of the oocyte-type 5S genes, both because the primary transcripts derived from well characterized genes have been analysed, and the degree of developmental inactivation of these 5S genes is exceptionally strong. Nuclei injected into oocytes do not undergo division²⁶, and the activation therefore applies to the same actual genes or molecules of

DNA as were previously inert. Our demonstration of 5S gene activation depends on the resolution of RNAs of identical size by gel electrophoresis in non-denaturing conditions, a method which is likely to be of considerable value in separating other groups of related RNAs such as low-molecular-weight nuclear RNAs³⁵.

The experiments reported here have shown that one can study the activity of genes in a regulated ('switched-off') condition. As far as we know, all cases in which purified genes as DNA have been tested for activity *in vitro* or *in vivo* result in the loss of the regulated state; for example both oocyte and somatic types of 5S DNA are fully active when injected into oocytes or added to somatic cell extracts^{4,10,11}. This unique ability to observe regulated gene expression derives from the fact that in whole nuclei, as used in this study, genes are in their natural chromosomal environment. Thus the genes are still complexed with protein or other molecules with which they are naturally associated. The possibility that the developmental regulation of genes may require larger segments of DNA than are at present convenient to clone, or the cooperation of parts of the genome entirely unlinked to the genes being studied is easily accommodated by our system.

Our experiments give only indirect information about the nature of the activating effect of oocytes and hence about the developmental inactivation which is reversed. (1) Treatment with 0.35 M NaCl, which dissociates only a minority of chromosomal proteins²⁹, has nearly as much reactivating effect as 2 M NaCl and detergent which removes most of the molecules from DNA. This emphasizes again the lability of the developmental inactivation of 5S genes. We do not know whether NaCl activation involves the same mechanism as the effect of activating oocytes on untreated nuclei. (2) The 5S DNA-binding protein which promotes transcriptional activity *in vitro*^{31,33} did not cause activation when tested by injection. Gene activation therefore seems unlikely to result from the mere presence of this protein in activating oocytes and its absence or low concentration in non-activating oocytes. This protein has not yet been shown to be able to discriminate between oocyte- and somatic-type 5S genes in its transcription-promoting effect.

The surprising existence of certain females which have non-activating oocytes, as well as others which have activating oocytes, was at first disturbing, but may eventually be providential; we can now prepare extracts of activating or non-activating oocytes and inject these with somatic nuclei into oocytes of the opposite type, with a view to fractionating the active extract.

We thank S. Whytock and R. Wells for assistance, Drs D. Brown, R. Roeder, K. Nishikura and E. De Robertis for communicating unpublished results, Dr J. Gottesfeld for providing *X. laevis* kidney tissue culture cells, and Drs D. Brown, R. Laskey, D. Melton, B. Meyer, G. Partington, J. Wells and M. Wickens for helpful discussions and comments on the manuscript. During part of this work, L.J.K. was a postdoctoral fellow of the Helen Hay Whitney Foundation.

Received 18 September; accepted 2 December 1980.

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Cruciform structures in supercoiled DNA

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Specific sites in supercoiled pVH51 and pBR322 DNAs are cleaved by single-strand specific nucleases. These sites are in the loops of potential cruciform structures.

CRUCIFORMS have been favourite hypothetical recognition sites on DNA since they were first postulated¹. The observation that several regulatory sites contain varying lengths of inverted repeat sequences encouraged these speculations². If a cruciform existed *in vivo*, even transiently, it could be stabilized by a suitable protein. In supercoiled DNA, however, the necessary free energy could be provided by supercoiling and relatively stable cruciform structures might exist. The thermodynamic properties of superhelical DNA and potential cruciform structures have been studied³⁻⁵.

This article describes strong evidence supporting two naturally occurring cruciform structures in supercoiled DNA.

Cleavage of supercoiled DNA by single-strand specific endonucleases

Figure 1 shows the rates of cleavage of supercoiled pVH51 DNA by S₁ nuclease⁶ (a) and the T₇ gene 3 product⁷⁻⁹ (b). In both cases, the supercoiled DNA (form I) is converted to the nicked-circular DNA (form II), which is then converted into the linear form (form III). Further nicking events on form III eventually cause double-strand breaks and generate smaller molecules which appear as a smear on the gel. Figure 1a shows that when relatively high levels of S₁ nuclease were used, complete conversion of the supercoiled DNA to form II was observed after only a few seconds. After 8 min, almost all of the nicked DNA had been linearized and by 90 min most had been degraded to smaller pieces. When lower levels of the T₇ gene 3 product were used, the gradual conversion of the supercoiled DNA to form II was observed over 4 min. Using appropriate levels of enzymatic activity, each of the discrete steps of the reaction can be monitored. Overall, pVH51 DNA is cleaved by S₁ nuclease and the T₇ gene 3 product with similar rates for each step.

Although these data permit a kinetic evaluation of the conversion of supercoiled DNA to linearized segments, they cannot provide information on the position-specificity of the cleavage, as both forms II and III are obligatory intermediates in the reaction.

Mapping of cleavage sites on pVH51 DNA

To determine if the nucleolytic cuts in supercoiled pVH51 DNA were at specific or random loci, the DNA was treated with each single-strand specific endonuclease and then specifically cleaved with a restriction enzyme. If the cleavage was the result of nicking at random sites, identical restriction patterns would be obtained for the single-strand specific nuclease-treated and untreated DNA. On the other hand, if linearization occurred at specific sites, the restriction pattern of the single-strand specific nuclease-treated DNA would show the loss of one (or more) fragment(s) and the appearance of new fragments with the same total length.

The results of these experiments are shown in Fig. 2. pVH51 DNA was incubated with S₁ nuclease until approximately one-half of the DNA was linearized (Fig. 2a, lane 2). (The reaction was not carried further so as to prevent the degradation that

accompanies extensive linearization (see below).) When the S₁-treated and untreated DNAs were digested with *Hae*III, the gel analysis showed the typical pattern expected from this digest (ref. 10 and refs therein) (Fig. 2b). However, as shown in lane 2, the intensity of the 610-base pair band of the DNA that had been treated with S₁ nuclease was reduced by approximately 50% relative to the undigested control and, in addition, two new bands appeared. The sizes of the two new fragments were 340 and 270 base pairs and their sum (610 base pairs) equalled the size of the fragment whose intensity was reduced. This indicated that S₁ nuclease cleaves pVH51 DNA very specifically at a position inside the 610-base pair *Hae*III fragment.

When pVH51 DNA was linearized with the T₇ endonuclease and then restricted with *Hae*III, the same two new bands appeared at 340 and 270 base pairs (Fig. 2c, lane 5), indicating that this enzyme also cleaves pVH51 DNA at the same specific position as the S₁ nuclease. Furthermore, when analysed by a double digest with *Hae*III and *Hind*II, *Hind*II cleaved the 610-base pair fragment to the 510- and 100-base pair products expected from the known restriction map. The 340-base pair fragment was also cleaved to two new fragments 240- and

Table 1 Perfect inverted repeats

DNA	Base at centre of symmetry	No. of bases in stem	No. of bases in loop
pVH51	951.0	9	7
	1,009.0	9	5
	1,514.0	13	5
pBR322	3,220.5	10	6
	3,065.0	11	3
G4	2,671.5	8	15
	3,982.0	8	5
	2,571.5	9	0
ΦX174	3,008.5	8	8
	3,965.0	9	3
fd	4,698.5	8	12
	5,780.0	8	11
	5,803.0	8	3
	92.5	9	14
	1,548.5	9	4
SV40	3,331.0	9	5
	668.5	8	8
	2,026.0	8	7
	2,651.0	8	11
	5,092.0	8	4
	5,161.0	13	1

The DNA sequences of pVH51 (ref. 11 and J. Tomizawa, personal communication), pBR322¹⁶, G4²⁸, ΦX174²⁹, fd³⁰ and SV40³⁰ were searched by computer for perfect inverted repeat sequences (only A+T and G+C pairs with no interruptions in the stems) which contain a maximum of 16 nucleotides in the loop regions. As expected, the number of inverted repeats decreases sharply as the stem length increases. Many perfect inverted repeats were found for all DNAs but only those with the two longest stem lengths are listed. Approximately 75% (2,694 base pairs) of the pVH51 sequence was available for this search.

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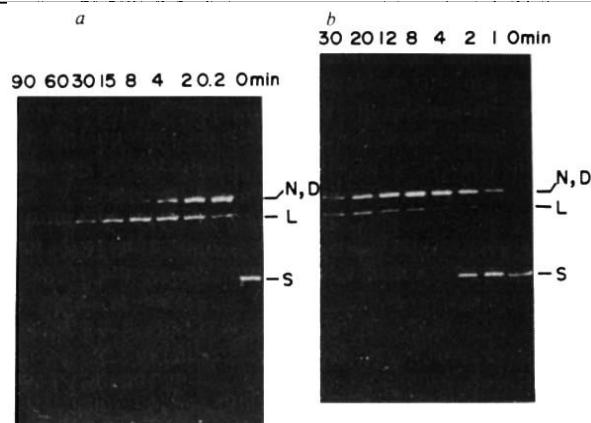


Fig. 1 Effect of single-strand specific endonucleases on supercoiled DNA. *a*, S_1 endonuclease; *b*, T_7 endonuclease. Reactions with S_1 nuclease were carried out in 40 mM sodium acetate buffer, pH 4.6, 0.05 M NaCl and 1.0 mM $ZnSO_4$. pVH51 DNA (5 μ g) was reacted with 8 U of S_1 nuclease (specific activity 120,000 U per mg protein) in a final volume of 0.3 ml. The characterization of this preparation of S_1 nuclease has been described²¹. Reactions with T_7 endonuclease were carried out in a buffer containing 50 mM Tris-HCl, pH 7.6, 0.1 mM dithiothreitol, 10 mM $MgCl_2$ and 2.5 mM spermidine (hereafter called T_7 buffer). pVH51 (4 μ g) was reacted with 6 U enzyme (specific activity 12,000 U per mg protein) in a final volume of 0.3 ml. T_7 endonuclease is the product of bacteriophage T_7 gene 3 (refs 7-9) and was purified from T_7 -infected *E. coli* cells. The final preparation exhibited one major band, constituting at least 80% of the protein, on SDS-polyacrylamide gels (N.P. and R. D.W., unpublished results). One unit of activity is defined as the amount of enzyme necessary to convert 1 μ g of supercoiled DNA into a mixture of nicked-circular and linear forms in 10 min in the conditions described above. All enzymatic reactions were carried out at 37°C. Aliquots (30 μ l) were removed, made 10 mM in EDTA at the indicated times and the mixtures were electrophoresed on 1% agarose gels and stained as described previously²². The positions of the supercoiled (S), linear (L), nicked (N) and dimer (D) DNA molecules are indicated. pVH51 DNA²³ was prepared as described previously²².

100-base pairs long (Fig. 2c, lane 2). Thus, the unique linearization site must be located 240 ± 4 base pairs from the single *Hind*III site of pVH51 DNA, which is inside the *Hae*III 610-base pair fragment.

The presence of a specific linearization site on pVH51 DNA was demonstrated with a nuclease-treated DNA preparation that contained approximately half form II and half linear molecules. At that ratio, nonspecific DNA degradation is minimal. However, if the nuclease reaction was allowed to proceed further to the point where all form II molecules were linearized, roughly 50% of the DNA was degraded to linear molecules of smaller than full-length size (Fig. 1a). On restriction of such a reaction mixture, the degraded molecules appeared on polyacrylamide gels as a background of numerous faint bands or even as a smear, depending on the progress of the reaction (data not shown). This indicates that, in addition to the highly specific linearization reaction, these single-strand specific nucleases are capable of degrading duplex DNA at a lower rate, by cleavage at less specific sites.

A 31-base pair inverted repeat sequence spans the linearization site

The discrete sizes of the 340-, 270- and 240-base pair fragments (Fig. 2) suggest that linearization results from nicks on the complementary strands at rather precise locations. If cleavage actually occurred at unique positions, it should be possible to end-label both strands at the cleavage sites and determine their nucleotide sequence. On the other hand, if linearization exposed more than one nucleotide at each end, labelling would not be specific and the sequencing gel would be impossible to read.

pVH51 DNA was linearized with S_1 nuclease, end-labelled and sequenced as described in Fig. 3 legend. Clear patterns were observed on the sequencing gel indicating that cleavage occurred at unique positions on each strand. Comparison of these sequences with the published sequence of pVH51 DNA (ref. 11, and J. Tomizawa, personal communication) revealed that the unique ends generated by S_1 nuclease were located exactly opposite each other on the two strands. Furthermore, the cleavage site was located 238 base pairs from the single *Hind*III site of pVH51, in full agreement with the mapping data obtained with both the S_1 and T_7 nucleases.

Figure 3 shows the DNA sequence around the site of linearization. The remarkable feature of this sequence is an unusually long (13 base pairs) and perfect (no interruptions or G·T pairs) inverted repeat. The sequence can be shown in the form of a cruciform structure having double-stranded stems that are 13 base pairs long and single-stranded loops consisting of five nucleotides. The nicking site on each strand is located in the middle of each loop.

From these data, the following mechanism is proposed for the position-specific linearization of pVH51 DNA by the single-strand specific nucleases. The enzymes recognize a unique structural feature and cleave one strand, converting the supercoiled molecule into form II. Subsequently, the enzymes cleave the complementary strand exactly opposite the nick at a slower rate. At present, it is uncertain whether one of the complementary strands is cleaved first preferentially. Studies on the nonspecific nicking of PM2 DNA and Φ X174 RFI DNA by the

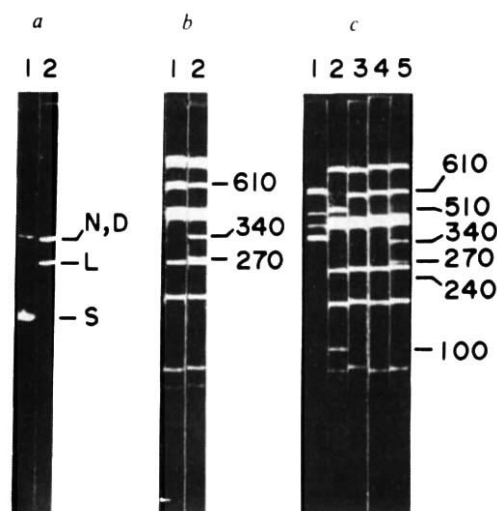


Fig. 2 Mapping of the cleavage site. *a*, Linearization of supercoiled pVH51 DNA with S_1 nuclease. Lane 1, 0.2 μ g pVH51 DNA; lane 2, 0.4 μ g pVH51 DNA reacted with S_1 nuclease. pVH51 DNA (60 μ g) in a final volume of 1.0 ml was reacted with S_1 nuclease (15 U) at 37°C for 5 min. The reaction mixture was extracted with phenol, followed by ether, and dialysed against 10 mM Tris-HCl, pH 8.0, 0.05 mM EDTA overnight. Aliquots of this mixture were used for subsequent reactions with restriction enzymes. Samples were analysed on 1% agarose gels. *b*, Mapping the S_1 cleavage site. Lane 1, 2 μ g pVH51 DNA digested with *Hae*III; lane 2, 2 μ g pVH51 digested with S_1 nuclease followed by *Hae*III. *c*, Mapping the T_7 endonuclease site. Lane 3, pVH51 DNA (2 μ g) reacted with *Hae*III for 3 h; lane 4, pVH51 reacted with *Hae*III for 3 h followed by T_7 nuclease for 30 min; lane 5, pVH51 reacted with T_7 nuclease for 30 min, followed by *Hae*III for 3 h; lane 2, pVH51 reacted with T_7 nuclease for 30 min, followed by *Hae*III and *Hind*III for 3 h; lane 1, a mixture (1 μ g) of restriction fragments (365–610 base pairs in length; isolated for another purpose from pVH51 DNA by RPC-5 column chromatography²⁴) incubated with T_7 nuclease for 3 h. All reactions were carried out in 40 μ l at 37°C in T_7 buffer. The second enzyme was added in a small (2 μ l) volume. The sources and reaction conditions for all restriction enzymes used have been described^{10,22,24-26}. The analyses in *b* and *c* were on 5% polyacrylamide gels. The sizes¹⁰ (in base pairs) of selected restriction fragments are indicated.

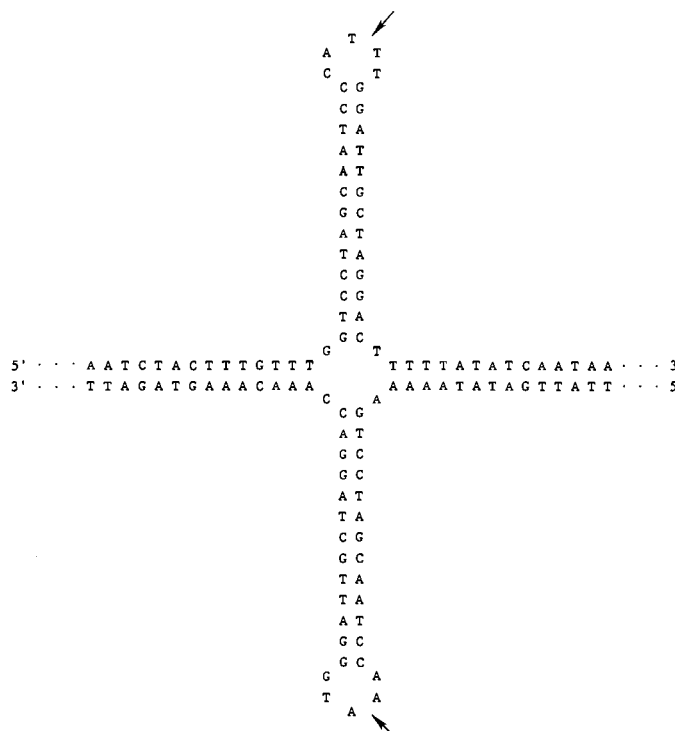


Fig. 3 pVH51 DNA sequence around the site cleaved by the single-strand specific endonucleases, drawn in cruciform structure. The positions of cleavage for each strand are indicated by arrows. pVH51 DNA was linearized with S_1 nuclease as described in Fig. 2 legend. Following treatment with bacterial alkaline phosphatase, the DNA was labelled with [γ - 32 P]ATP, restricted with *Hae*III and fractionated on 5% polyacrylamide gels. Autoradiography revealed that 80–90% of the radioactivity was associated with the 340- and 270-base pair fragments. These fragments were eluted from the gel and sequenced as described²⁷ with some minor modifications²⁵. Segments of both strands were sequenced and were in complete agreement with the published sequence¹¹.

Neurospora nuclease have shown that the first nick is not strand specific^{5,12}. Likewise, it is uncertain whether one enzyme molecule cleaves both strands or whether the enzyme dissociates from the substrate after the first nicking event.

As described above, mapping studies on the site of cleavage with the T_7 gene 3 product demonstrated that both this enzyme and S_1 nuclease cleave at an identical (± 4 base pairs) site. As the T_7 endonuclease was assayed at pH 7.6 in the presence of 10 mM $MgCl_2$, the possibility that the position-specific cleavage is induced by the unusual environment of the S_1 reactions (pH 4.6, 1 mM $ZnSO_4$) can be ruled out.

Supercoiling is necessary for site-specific cleavage

To determine the effect of supercoiling on the linearization rate, pVH51 DNA containing varying numbers of negative, superhelical turns was treated with the T_7 nuclease. The supercoil density and the appearance of linear molecules were monitored on agarose gels. Figure 4 shows that topoisomers within a higher density of supercoil turns are better substrates than the partially relaxed molecules. As the population of substrate DNAs with less supercoil turns increases (Fig. 4b), the amount of the linear molecules which are formed during the cleavage reaction decreases (Fig. 4a). In fact, the decrease in intensity of the band corresponding to linear molecules (Fig. 4a) closely parallels the decrease in intensity of the band of the most supercoiled molecules (Fig. 4b). In addition, partially relaxed molecules were much more resistant to the T_7 nuclease (Fig. 4a). To verify that the linear molecules observed in Fig. 4a resulted from site-specific cleavage, the DNA was restricted with *Hae*III and fractionated on polyacrylamide gels (Fig. 4c). The 340- and

270-base pair fragments were observed, as expected, and their intensities paralleled the intensity of the linear molecules observed in Fig. 4a.

Likewise, DNA restriction fragments, containing the sequence which is specifically cleaved in supercoiled DNA, are not cleaved in parallel conditions. Figure 2c, lane 4, shows that when pVH51 DNA was first restricted with *Hae*III and then treated with the T_7 nuclease, the 340- and 270-base pair fragments were not produced from the 610-base pair fragment. The same lack of specific cleavage was also observed when a mixture of restriction fragments including the 610-base pair fragment was incubated with the T_7 enzyme for prolonged times (Fig. 2c, lane 1). Hence, the DNA must be in a supercoiled, not relaxed or linear, conformation to be cleaved specifically by these single-strand specific nucleases.

Previous studies with the *Neurospora* endonuclease⁵, mung bean nuclease⁵, S_1 nuclease¹³ and *Alteromonas espejiana* BAL 31 nuclease¹⁴ have also shown that supercoiled DNA is a preferred substrate for the initial nick (conversion of form I to II). In contrast, the rate of nicking of PM2 DNA by pancreatic DNase I is independent of the degree of supercoiling⁵.

Although a remarkably long, inverted repeat sequence exists around the cleavage site and the cuts are in the loops of the potential cruciforms, these data do not directly prove the presence of a cruciform structure. Two possible alternative recognition features (modified nucleotides and R-loops) have been considered and ruled out.

The presence of some types of modified nucleotides in pVH51 DNA specifically at the cleavage site could cause a partially disrupted DNA helix. To test this hypothesis, pVH51 DNA was grown in two different *Escherichia coli* host cells¹⁰, M0 and

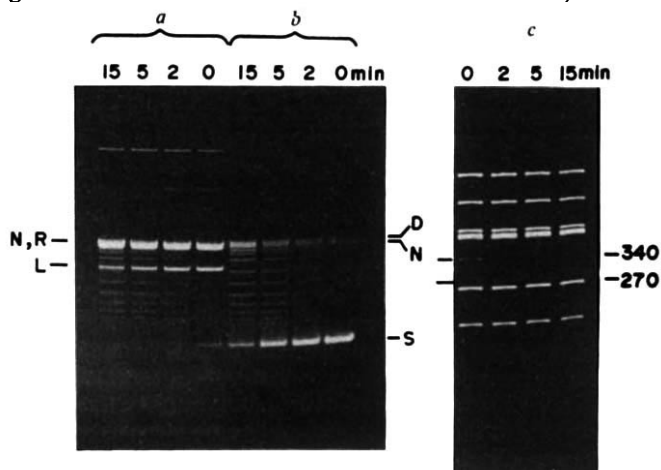


Fig. 4 Effect of supercoiling on the cleavage reaction. *b*, pVH51 DNA incubated with a nicking-closing enzyme for the indicated times; *a*, the partially relaxed DNA shown in *b* after incubation with T_7 endonuclease for 3 min; *c*, the partially relaxed and cleaved DNA shown in *a* after restriction with *Hae*III. pVH51 DNA (22 μ g) was reacted at 37 °C with 4 μ g wheat-germ topoisomerase in 60 μ l T_7 buffer containing 30 mM NaCl. At the indicated times, each reaction mixture was placed at 72 °C for 10 min to inactivate the enzyme and the solution was made 0.1 M with NaCl. Aliquots (10 μ l) were then removed for electrophoresis on 1% agarose (*b*). T_7 endonuclease (1.2 U) was then added and the mixtures incubated at 45 °C for 3 min, followed by 10 min at 72 °C to inactivate the enzyme. Aliquots (10 μ l) were removed for electrophoresis on 1% agarose gels (*a*). *Hae*III (2 U) was then added to each mixture and incubated for 3 h at 37 °C before electrophoresis on 5% polyacrylamide gels (*c*). The latter designations are as in Fig. 1 and R indicates the positions of relaxed, covalently closed, circular DNA. The wheat-germ DNA topoisomerase I was isolated and purified to approximately 60% purity by W. Dynan, J. Jendrisak, D. Hager and R. Burgess (unpublished method; gift of R. Burgess). This enzyme is free of nuclease activity and the products of a complete reaction are covalently closed, circular DNA without detectable amounts of nicked molecules. The faint bands on the top portion of *a* correspond to the form II-dimer and linear-dimer forms.

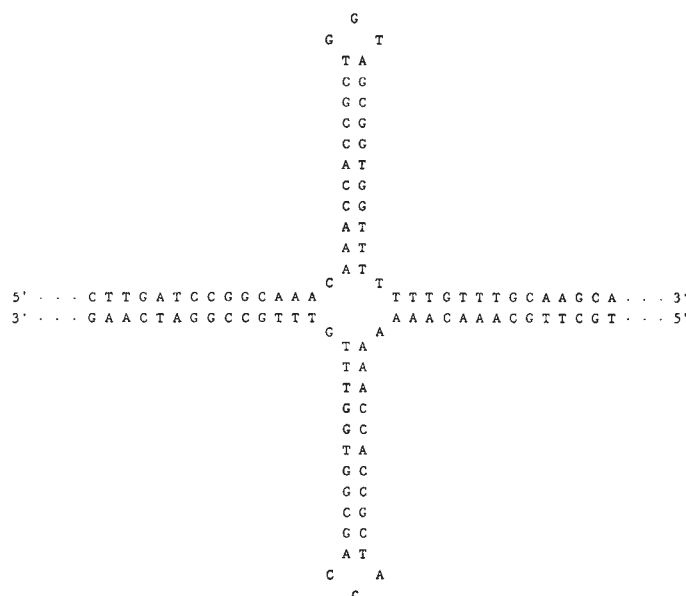


Fig. 5 pBR322 DNA sequence around the site cleaved by the single-strand specific endonucleases, drawn in a cruciform structure. The sequence is taken from ref. 16.

C600 SF8, the latter strain lacking a known modification system. Identical specific cleavages were found for both DNAs.

The second possibility is that an R-loop¹⁵ might be present at the linearization site in the supercoiled DNA. This hypothesis was also eliminated when heat-denatured pVH51 DNA (which loses its R-loops¹⁵) produced the same results as native DNA.

Site-specific cleavage of supercoiled pBR322 DNA

If specific cleavage sites existed on supercoiled molecules other than pVH51 DNA, a comparison of their sequences might provide evidence as to the nature of the structural features necessary for recognition.

Supercoiled pBR322 DNA was linearized with the *T₇* endonuclease and subsequently restricted in the manner described for pVH51. Two new bands appeared in the restriction patterns of the *T₇* nuclease-treated DNA, indicating the presence of a specific cleavage site. The use of several restriction enzymes (*Hind*II, *Hind*III, *Taq*I, *Hinf*I and *Hae*II) allowed rather precise mapping of the cleavage site at position $3,061 \pm 5$ base pairs of the published sequence of pBR322¹⁶. A *Taq*I digest of pBR322 DNA that had been treated with *S₁* nuclease produced the same result.

Inspection of the DNA sequence revealed the presence of an inverted repeat around the cleavage site with a centre of symmetry at position 3,065. This repeat could be drawn into a cruciform configuration having double-stranded stems that are 11 base pairs long, and single-stranded loops consisting of three nucleotides (Fig. 5). The position of the loops corresponds to the position of cleavage, within experimental error. Therefore, as with pVH51, site-specific cleavage of pBR322 occurs at a position surrounded by an unusually long, inverted repeat sequence. Comparison of the DNA sequences around the cleavage sites in pVH51 and pBR322 revealed no significant homology that might constitute potential recognition sequences. Moreover, the requirement for superhelicity and the fact that two unrelated enzymes with no known sequence specificity cleave at the same site have already ruled out the possibility that the DNA sequence is recognized as a double-stranded helix. Instead, these results strongly support the notion that the supercoil pressure induces a tertiary (cruciform) structure which is maintained long enough for the single-strand specific nucleases to cleave at the single-stranded loops.

Absence of site-specific cleavage of other DNAs

Several other supercoiled DNAs were screened for position-specific cleavage. The replicative forms of G4, Φ X174, fd and M13 DNAs, as well as SV40 DNA, were linearized with the *T₇* nuclease; *S₁* nuclease linearization on SV40 and Φ X174 RFI DNAs was also studied. The nicking and linearization rates were comparable with the rates observed with pVH51 DNA (data not shown). Each DNA was then cleaved with at least two different restriction enzymes, in the manner described for pVH51 DNA, to ensure that the potential linearization site(s) did not overlap with a restriction site. In some cases, several bands were observed, indicating simultaneous linearization at many sites, but no unique bands that would allow unequivocal mapping of a cleavage site were found. Similar results have been published for the cleavage of Φ X174 RFI DNA by the *Neurospora* nuclease¹². Therefore, G4, Φ X174, fd and M13 DNAs do not possess the unique structural features which, in the same conditions, lead to site-specific cleavage of pVH51 and pBR322 DNAs.

As the linearization sites on both pVH51 and pBR322 are in the middle of unusually long, inverted repeats, the lack of site-specific cleavage of G4, Φ X174, fd and M13 DNAs might be due to the absence of long, inverted repeats from the sequences of these molecules.

The published sequences of all six DNAs were searched by computer for inverted repeats and for A+T-rich regions.

Table 1 shows that pVH51 DNA contains the longest, perfect inverted repeat in the total of approximately 30,000 base pairs; this potential cruciform (Fig. 3) is the site of specific cleavage. The second longest repeat (13-base pair stem, one-base loop) was found in SV40 DNA. However, considering that a loop of less than three to four bases is sterically constrained¹⁷, the maximum stem size for a cruciform of this repeat is 11 base pairs. The next longest repeat (11-base pair stem, three-base loop) was found in pBR322 DNA and this potential cruciform (Fig. 5) is the site of specific cleavage.

It may be necessary for the inverted repeat sequence to be within an A+T-rich segment of much larger size to form a relatively stable cruciform. The single cleavage site in pVH51 is located in a unique, easily denaturing and unusually A+T-rich region¹⁸. Likewise, the specific cleavage site in pBR322 DNA is located in one of the two most A+T-rich regions in that DNA. In contrast, SV40 DNA was not specifically cleaved. The experimental and theoretical denaturation maps of this DNA reveal that the region surrounding the largest inverted repeat is not particularly A+T-rich, whereas the molecule contains three other very distinct easily denaturing regions¹⁹.

Supercoiling is known to generate locally single-stranded regions in double-stranded DNA molecules. These regions are easily denaturing stretches of DNA, due to their high A+T content². Our data suggest that cruciforms which exist in A+T-rich regions are the most stable. It is possible that the non-paired nucleotides in the A+T-rich region, which are in dynamic equilibrium with the duplex form, might facilitate the formation of a cruciform. Alternatively, if an inverted repeat is within a larger G+C-rich region, the duplex form of the DNA may be the most stable. According to this model, specific nucleases would cleave supercoiled DNA at specific sites if (at least transiently) stable cruciform structures could be formed within the most easily denaturing region. Inverted repeats present outside the most A+T-rich region would not readily form cruciforms and, therefore, would not be the first sites to be cleaved. On the other hand, if the most A+T-rich region did not contain inverted repeats, cleavage might still occur at random sites inside this region due to its single-stranded nature.

Accordingly, this model would predict that linearization of SV40 DNA by *S₁* and *T₇* nuclease would probably occur not at the inverted repeat but, instead, at the most easily denaturing regions. Previous studies have shown that *S₁* nuclease cleaves supercoiled SV40 DNA at a preferred region, which is located at 0.45–0.55 map units and coincides with an easily denaturing

region¹³. We observed that linearization of SV40 DNA by S₁ and T₇ nucleases was not reproducibly found at a specific site in the relatively low-temperature conditions which lead to position-specific cleavage of pVH51 DNA. In contrast, at high temperatures (72 °C), specific linearization was observed at position 4,540 ± 50 base pairs of the published sequence²⁰. This position corresponds to 0.55 map units, in the middle of one of the three most A+T-rich regions. Thus, these results are consistent with the above predictions.

In a special case, Gellert *et al.* (reviewed in ref. 2) constructed a completely inverted repeat DNA by recombinant techniques and showed that it could assume a cruciform structure.

Conclusions

We report here the first evidence supporting the existence of cruciform structures in naturally occurring DNA sequences. The supercoiled forms of two different DNAs (pVH51 and pBR322) contain specific cleavage sites for two endonucleases (S₁ and the T₇ gene 3 product) which are specific for single-stranded

regions. Both sites are in the centre of unusually long, inverted repeats and coincide with the single-stranded loops of potential cruciform structures. Five other DNA molecules (G4, ΦX174, fd, M13 and SV40), which lack long inverted repeats, are not cleaved specifically. The DNAs must be in a supercoiled form to be cleaved specifically; topoisomers with a larger number of supercoiled turns are cleaved more rapidly than those which are partially or totally relaxed. R-loop structures and one nucleotide modification system were eliminated as possible explanations. These results indicate that superhelicity induces tertiary (cruciform) structures which expose specific bases in single-stranded conformations.

This work was supported by NIH and NSF grants CA 20279 and PCM77-15033, respectively. We acknowledge the gifts of SV40 DNA by J. Mertz, the replicative forms of ΦX174, G4, fd and M13 DNA by G. Horn, and pBR322 DNA by W. Hillen. The computer programs used to search for inverted repeats and A+T-rich regions were developed by G. Horn and R. Littlewood, respectively.

Received 21 July; accepted 10 November 1980.

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LETTERS

Galactic γ and radio synchrotron radiation

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The galactic γ -radiation and radio synchrotron radiation contains information on the distribution of cosmic-ray particles, magnetic field and gas throughout the Galaxy. We report here a new all-sky survey of 408-MHz radio continuum which has been used to compare the distributions of radio and γ -ray emission, with the former convolved to the response function of the COS B γ -ray detector. The overall similarity of the longitude profiles suggests that the bulk of the γ -ray emission is from the interstellar medium rather than discrete sources. On a large scale in the galactic plane the two emissivities seem to be similarly distributed, although in detail the γ -ray emission is more clumpy.

γ rays with energies in the region of interest (~ 100 MeV) are produced by bremsstrahlung of cosmic-ray electrons of a few 100 MeV on the interstellar gas and from the decay of neutral pions, produced in the interactions with the gas of cosmic-ray nuclei with energies of a few GeV. (The inverse Compton interactions of cosmic-ray electrons are expected to give a much smaller contribution than these two processes except, perhaps, at high galactic latitudes.) If the cosmic-ray proton to electron

ratio is constant, the interstellar γ -ray emissivity is then proportional to the product of cosmic-ray intensity and total gas density. There is, of course, independent information on the distribution of interstellar gas in the Galaxy so that, in principle, the distribution of cosmic rays can be obtained (the work of the past decade on this problem is reviewed in ref. 1). In practice, there are large uncertainties because the important molecular hydrogen component of the gas has to be inferred from the distribution of CO, which is itself only known in certain regions. Also, mainly because of the rather poor angular resolution of the γ -ray detectors, there is uncertainty concerning the contribution of unresolved discrete sources to the apparently diffuse γ -ray emission along the galactic plane. Additional information may therefore be usefully sought from a comparison of the γ -ray distribution with that of radio synchrotron radiation. In the frequency range from 100 MHz to 1 GHz, where there is the best combination of good angular resolution and low contamination by thermal continuum radiation, the synchrotron radiation is produced by cosmic ray electrons of a few GeV. The synchrotron emissivity is proportional to the product of the cosmic-ray electron density and approximately the square of the magnetic field intensity (B^2). The large scale distribution of B^2 may in turn possibly be related to the distribution of interstellar gas. If, for example, the γ -ray and synchrotron emissivities were in a constant ratio then B^2 would be proportional to gas density irrespective of the variation of cosmic-ray density.

Comparisons between the γ rays and synchrotron radiation have been made by several authors (see refs 2–4). They have mainly considered γ -ray data, having rather low statistical accuracy, from the SAS 2 detector (8,000 γ rays) and the synchrotron radiation data were in a form which did not make the necessary convolution easy. We now use the new all-sky survey at 408 MHz of Haslam *et al.*^{5,6}. This is from observations at Effelsberg, Jodrell Bank and Parkes. The data, smoothed to a

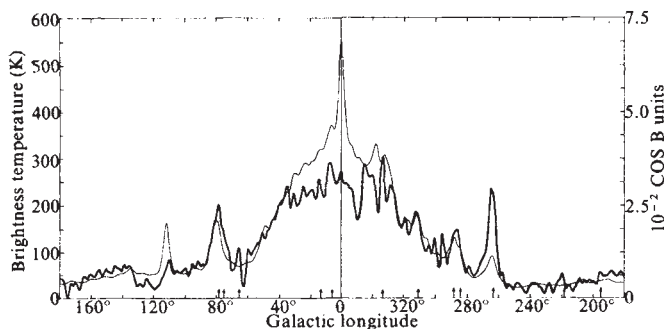


Fig. 1 Galactic plane profiles. Thick line and right-hand scale: distribution of 70 MeV to 5 GeV γ rays obtained by the COS B detector. The arrows show the longitudes of features corresponding to γ -ray 'sources'. Thin line and left-hand scale: distribution of 408 MHz continuum radiation convolved to the HPBW of the COS B detector.

common resolution of 51 arc min, are in the form of brightness temperatures at 20 arc min intervals of galactic latitude and longitude. It has been convolved and averaged appropriately for comparison with the COS B γ -ray distributions of Mayer-Hasselwander *et al.*⁷ based on 64,000 photons. We present here the comparisons with latitude and longitude distributions of γ rays with energy 70 MeV $< E < 5$ GeV. Comparison with the longitude distributions of γ -rays after division into three energy bands in this range leads to similar conclusions although the effects of statistical fluctuations are naturally greater.

Figure 1 shows the longitude distributions. The γ -ray intensities are given in units of 'on-axis' counts $\text{s}^{-1} \text{sr}^{-1}$ for the COS B detector (henceforth referred to as COS B units). The thick line shows the cut along the galactic plane ($b = 0^\circ$) of the contour map derived using the smoothing procedure detailed in ref. 7. This removes features smaller than the half-power beam width (HPBW) of the detector but not all the remaining features are necessarily statistically significant. For comparison, the 408 MHz data must be convolved to the beam profile appropriate to the γ -ray detector. Such a profile was constructed taking into account the energy dependence of the HPBW (from 7° at 70 MeV to 2° at 5 GeV) and the relative numbers of γ rays of a given energy in the COS B data: it has a HPBW of 3.5° and a longer tail than a gaussian distribution. The result of the convolution is given as the thin line curve in Fig. 1. We have chosen the cut at $b = 0^\circ$ for the comparison rather than the longitudinal variation of the average γ -ray intensity for $|b| < 5^\circ$, which is also given in ref. 7, because the former is affected less by the differing latitude distributions of the γ and synchrotron radiation. An indication of the statistical significance of the features in the γ -ray cut at $b = 0^\circ$ can be obtained from the quoted statistical errors on these average intensities. We deduce that, over one beam area, statistical fluctuations of about 0.15×10^{-2} and 0.2×10^{-2} COS B units are to be expected for the outer and inner halves of the galactic plane respectively. Thus, while many of the features in the outer half of the plane can be discounted most of those in the inner half are real.

The latitude distributions are shown in Fig. 2. The points are the intensities at 1° intervals in latitude averaged over the longitude intervals indicated. The lines are the 408 MHz brightness temperatures convolved and averaged appropriately. As the object is to compare the galactic radio and γ -ray emission the isotropic extragalactic contributions have to be removed. From the 408-MHz data a 6 K isotropic component has been subtracted. The COS B intensities are those above a background. This is the sum of an instrumental background, an extragalactic component, and the estimated galactic radiation at $b = 90^\circ$. Strictly this last component should be added back in. From the SAS 2 experiment⁸ for $E > 70$ MeV this is $\sim 2.2 \times 10^{-5} \text{ cm}^{-2} \text{ s}^{-1}$ which seems to correspond to about 0.6×10^{-3} COS B units. For the longitudinal profile this is negligible. For

the latitude profiles the addition would be just large enough to make all of the plotted points positive. Although the 408 MHz continuum radiation is predominantly non-thermal there is some thermal contribution. In the plane of the Galaxy $\sim 20\%$ of the extended emission is thermal. The scale height of the thermal emissivity is smaller than that of the non-thermal radiation by a factor of between 5 and 10, so that after convolution the contribution at $b = 0^\circ$ is no more than a few per cent. The broad peak in emission underlying the sources in the Cyg X region ($l \approx 80^\circ$) is predominantly thermal, however.

Let us now consider the latitude and longitude profiles. In Figs 1 and 2 the radio γ -ray intensities are plotted with the same arbitrary normalization ($100 \text{ K} \equiv 1.25 \times 10^{-2} \text{ COS B units}$). This makes the longitudinal profiles generally coincide (to within $\sim 35^\circ$ of the galactic centre). The most obvious difference between the profiles is that there is no strong γ -ray source at the galactic centre corresponding to the radio source Sag A. This gives a contribution of 150 K to the convolved radio profile at $l = 0^\circ$ but has no effect on the profile for $4^\circ < l < 356^\circ$. Two other features must be discounted. The peak in the radio at $l = 112^\circ$ is due to the supernova remnant Cas A ($l = 111.7^\circ, b = -2.1^\circ$); this is not a detectable source of γ rays. The peak in the γ rays at $l = 263.5^\circ$ is essentially all pulsed emission from the Vela pulsar while the corresponding peak in the radio is unpulsed radiation from the Vela supernova remnant.

On the south side of the Galaxy the steps at $285^\circ, 310^\circ$ and 330° , usually regarded as evidence of spiral structure, are reproduced on both distributions while on the north side there are no steps in either distribution between 40° and 60° where one might expect the spiral arms to reappear. Both the distributions have a broad minimum at $l = 240^\circ$. The rather close resemblance of the two profiles (the discrepancy within 35° of the galactic centre is discussed below) supports the proposition that the γ -ray emission, like the synchrotron radiation, is predominantly produced in the interstellar medium rather than being due to discrete sources. It is possible, of course, that such a distribution of emission could be produced by discrete sources but their number density would have to follow rather closely the variation of the product of cosmic-ray density and the square of the galactic magnetic field strength. There does, however, seem to be more structure in the γ radiation than in the radio smoothed to the same HPBW even after statistical fluctuations are taken into account. To investigate the clumpiness of the γ -ray emissivity further more detailed data than that currently published are required. Not only the maxima but also the minima in the γ -ray distribution are of interest in this context. It is possible to account for the minimum at $l = 62^\circ$ in terms of interstellar production. This dip is almost certainly real although its sharpness may be the result of fluctuation. In this direction there is the longest line of sight to the nearest spiral arm in the whole galactic plane. When the Perseus arm is eventually reached at a distance of 10 kpc the scale height of the gas subtends an angle much less than the beam size and its contribution to the γ -ray intensity is much reduced. The effect will be less marked for the synchrotron radiation which has a considerably larger scale height.

Catalogues of γ -ray sources have been produced from the COS B data. A source is defined as a significant excess of counts compatible with the instrument point spread function so that a feature extending to $\sim 1^\circ$ is not distinguished from a point source. The second COS B catalogue¹⁰ contains 22 sources at low galactic latitudes. The longitudes of the 12 which contribute to features on the longitudinal profile are indicated by arrows on Fig. 1. Apart from the Vela pulsar there is no certain identification of any of these with a radio source but five features are coincident in position with radio sources strong enough to give features on the convolved radio profile. 2CG075+00 and 2CG078+00 are in the Cyg-X region. 2CG284-00 and 2CG288-00 are in the Carina spiral arm. In both regions there are various radio sources with which they might be identified including supernova remnants-OB associations (SNOBs)¹¹. The fifth source 2CG006-00 is perhaps the prime candidate for a

SNOB, being positionally coincident with the bright supernova remnant W28 and the HII region RCW146 both of which appear to be at a distance of 1.6 kpc from the Sun. In the direction of increasing longitude from the peak due to this source the next four γ -ray peaks all have corresponding features in the radio profile. The completely different behaviour of the profiles on the other side of the galactic centre suggests, however, that this is a chance effect.

Comparison of the latitude profiles in Fig. 2 shows that the synchrotron emission is much broader in extent than the γ radiation. This is true for all longitude ranges except $145^\circ < l < 175^\circ$. In the inner part of the Galaxy, as would be expected for production in the interstellar gas, the true latitude distribution of the γ radiation is masked by the HPBW of the detector. Taking the profiles in turn one sees that in Fig. 2*b* the North Polar Spur enhances the synchrotron emission at positive latitudes but not the γ radiation. In Fig. 2*c* the blackened area shows the contribution from the bright radiogalaxy Cyg A, which is not a γ -ray source. The contribution of Cas is shown similarly in Fig. 2*d*. The γ -ray peak in Fig. 2*f* is due to the bright source 2CG195+04. There is no significant radio emission associated with it. In Fig. 2*k* the peak in the γ -ray profile is due to the Crab pulsar. The γ rays are pulsed while the radio emission (shaded) is from the supernova remnant.

The latitude profiles show that the γ -ray emissivity is not proportional to the synchrotron emissivity in three dimensions. There could still be, however, an overall proportionality between the two emissivities in the galactic plane. The deficit in the γ -ray profile within 35° of the galactic centre does not preclude this as there are two geometrical effects that must be considered. The first is a manifestation of the differing scale heights of the γ and synchrotron radiation. The reduction in the recorded intensity due to the angular width of the emissivity being much less than the beam size of the detector is larger for the narrower γ -ray emission and increases with distance. The second is the effect of a regular component of the galactic magnetic field. In the neighbourhood of the Sun a regular component of the magnetic field is observed lying in the direction of galactic rotation and of the same order of magnitude as the fluctuating component. If this azimuthal field extends throughout the Galaxy there is an enhancement in the synchrotron radiation due to its being viewed perpendicularly as the line of sight approaches $l = 0$. The distribution of synchrotron emissivity in the galactic plane derived from the full resolution synchrotron profile by an unfolding technique in which the ratio of regular to irregular field is treated as a free parameter is to be reported elsewhere¹². A good fit is obtained for a ratio of unity and for an emissivity that peaks at a distance of 3.6 kpc from the

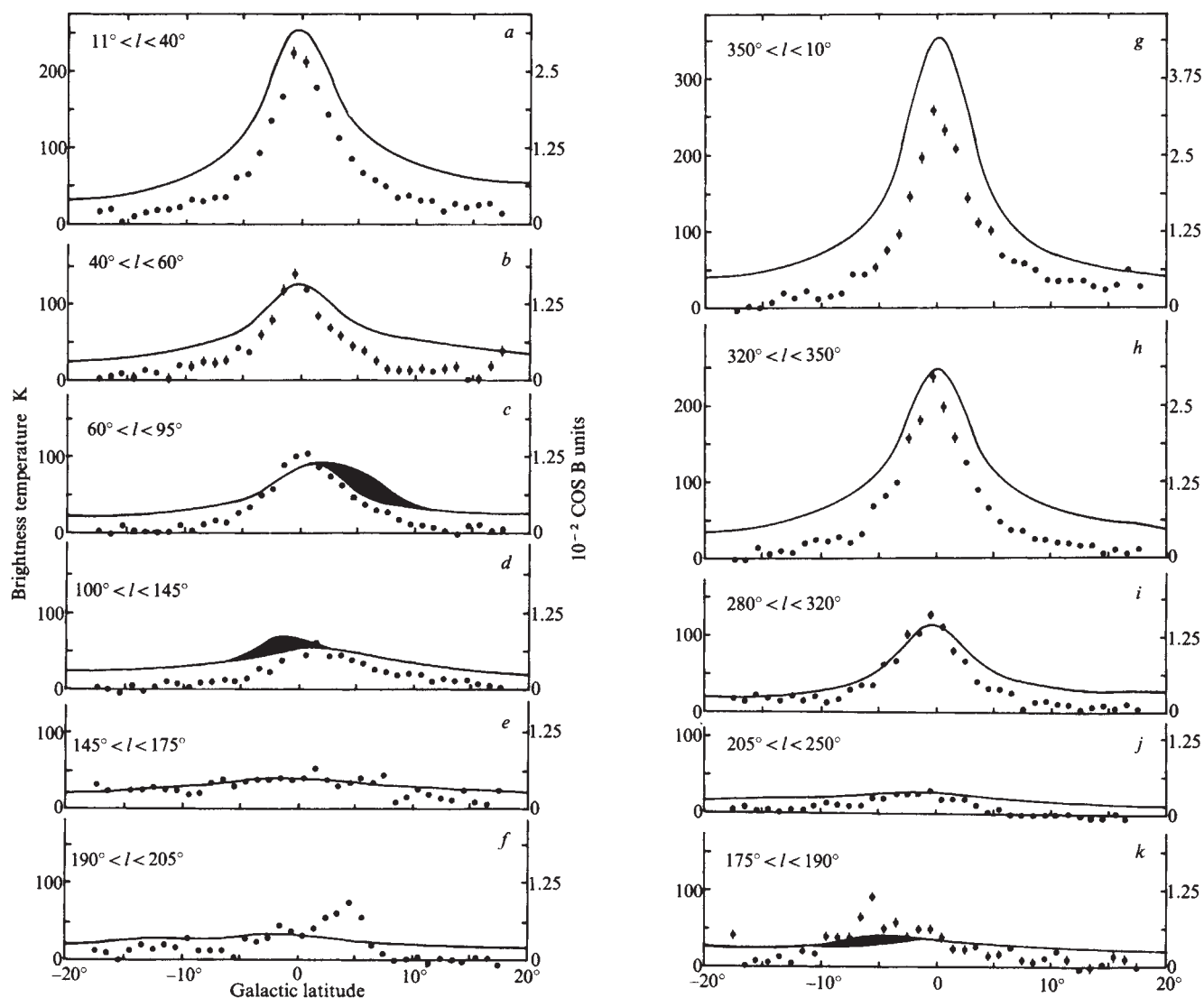


Fig. 2 Galactic latitude distributions. ●, γ -ray intensities averaged over the indicated longitude ranges. Statistical errors are shown when larger than the points. Lines, 408 MHz intensities correspondingly convolved and averaged.

galactic centre and is low within this distance. If we predict the γ -ray profile assuming that the emissivity in the plane mirrors this synchrotron emissivity but that its scale height is that of the gas, a reasonable fit to the observations is obtained. If the two emissivities are in constant ratio the implication is that B^2 is proportional to gas density on some suitably large scale in the plane of the Galaxy. Developing instabilities would extend the magnetic field out of the plane and destroy the proportionality there.

Received 2 September; accepted 5 December 1980.

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Laser-radar measurements in southern England of aerosols from Mount St Helens

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Material from the eruption of Mount St Helens (46°N , 122°W) has been detected at lower stratospheric heights using a laser-radar system at Winkfield (51°N , 1°W). The observation of a distinct layer of ~ 1 km thickness near 15.5 km altitude for several days in early June is consistent with the west-east transport of material from above the eruption site expected at this height and also indicates a significant northward spread of the material. In late July less pronounced layers were observed at 17–18 km and 22 km at which level the direction of motion is less certain.

The laser incorporated in the radar system operated near a wavelength of $0.6\ \mu\text{m}$ and produced $1\ \mu\text{s}$ pulses at a repetition frequency of $1\ \text{s}^{-1}$. The receiver included a 0.8-m diameter newtonian telescope and operated in a photon-counting mode, the return signals being recorded in channels corresponding to 1 km height intervals. The inclusion of a neutral density filter in the receiver and the restriction of the laser energy to a very low value ($< 20\ \text{mJ}$) ensured that the photomultiplier was not overloaded by returns from low altitudes.

The results obtained near the end of May and during June are presented in Fig. 1. These show the variations with height of the range-corrected backscattered signal and standard error for ~ 1 h of observations on each of four nights. In each case the corresponding height variation expected for the Rayleigh-scattered signal is shown, as derived from molecular densities obtained from radiosonde data for Crawley (51°N , 0°W) provided by the Meteorological Office, Bracknell, and fitted to the observations at the lower heights. The tropopause heights indicated were from the same data. The deviations from the Rayleigh scattering results on 27 May are consistent with other measurements at Winkfield before the eruption¹. Note the strong scattering from heights of ~ 15.5 km during the nights of 6 and 7 June; this feature was observed consistently between 4

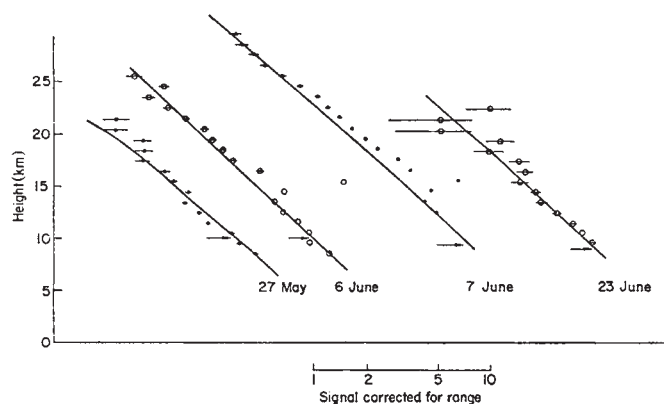


Fig. 1 Variations with height of the range-corrected signals from soundings at vertical incidence during four nights. The smooth curves represent the corresponding variations expected for Rayleigh scattering as computed for molecular densities derived from radiosonde measurements which also provided the tropopause heights denoted by arrows.

and 9 June, adverse weather conditions interfering with measurements on either side of these nights. In addition, small enhancements are shown over the height range 16–24 km on the 7 June. Balloon-borne observations² of scattered sunlight in southern France on 5 June also showed the presence of an enhanced aerosol layer at a height near 15.5 km. The presence in the eastern US on 22 May of layers of material between 12 and 16 km from the Mount St Helens eruption was recorded with the NASA Langley airborne laser radar system³. Furthermore, the absence of anomalous scattering at Winkfield on 27 May was consistent with the location of the 14–16 km material at $\sim 30^\circ\text{W}$, as observed³ with the satellite-borne Stratospheric Aerosol and Gas Experiment (SAGE). As indicated in Fig. 1, no major scattering was observed at Winkfield during the second half of June.

The approximate arrival date, relatively short duration and the location near 15.5 km of the layer observed at Winkfield are consistent with the transport of the layers observed previously in the eastern US by the west-east winds expected at these levels during May and June⁴. From an examination of the mean wind for the 100 mbar level, as derived by the Meteorological Office from radiosonde data, it is considered that a significant spread of the material towards the north must also have occurred. Unusual twilight effects following previous volcanic eruptions^{5,6} showed that the dust injected can spread over very large areas within about 2 months of the events. Furthermore, balloon-sonde⁷ and laser-radar observations^{8,9} revealed that the maximum enhancements of material extended over a considerable part of the 15–20 km altitude range, and continued for many months after the eruptions. Small enhancements in scattering have been observed from levels near 18 and 22 km during nights in the period 21–25 July, the only observable nights during that month.

We thank the Meteorological Office, Bracknell, for radiosonde data and information on winds at the 100 mbar level.

Received 26 September; accepted 17 December 1980.

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Similarities in amorphous and crystalline transition metal-metalloid alloy structures

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In many amorphous and glassy systems the local structure seems to be essentially the same in amorphous and crystalline phases. It thus becomes possible to define a 'local structural unit', such as the SiO_4 group in silicates. Determination of the structure then reduces to the problem of describing how the local units are connected and packed in the medium-range structure. There is mounting evidence that alloys of transition metals with metalloids are more adequately described by arrangements of stereochemically-defined structural units than by alternative descriptions in terms of random packing of atoms. There are three phases, Fe_3C , Ti_3P and Fe_3P , which may serve as models of local structural units. These lattices differ to a small degree in the symmetry of the metalloid nearest-neighbour shell but there are major differences in the arrangement of local structural units. Although the experimental evidence is limited, it seems that the distinctive features of the local and medium-range topologies of the crystalline phases are represented in the structures of each of the corresponding glasses.

Models for transition metal-metalloid (T-M) glasses have been constructed^{1,2} by randomly connecting local structural units—trigonal prisms consisting of six T atoms centred on M atoms, Fig. 1, using the edge-sharing arrangement found in the cementite Fe_3C lattice. Crystalline alloys with small metalloid radius ratios, such as Pd-Si, Ni-B and Co-B, have this structure. Calculations based on the model agree well with high resolution measurements of neutron scattering from amorphous Pd-Si alloys^{3,4}, giving a quality of fit at least equal to that of any other model. Moreover the model provides a good representation of the partial pair correlation functions for this alloy. Whether this type of model adequately describes other transition metal-metalloid glasses is particularly relevant for systems such as Ni-P or Fe-B in which crystalline T_3M phases are isostructural with Fe_3P or Ti_3P lattices respectively.

A relatively broad distribution of T-M bond lengths in Fe_3C structures, Fig. 2, reflects the distinction between distances from M to vertex or capping T atoms. This is particularly noticeable when M is small and T large, for example, in Pd_3B , where the trigonal prism is almost undistorted. The metalloid radius ratio

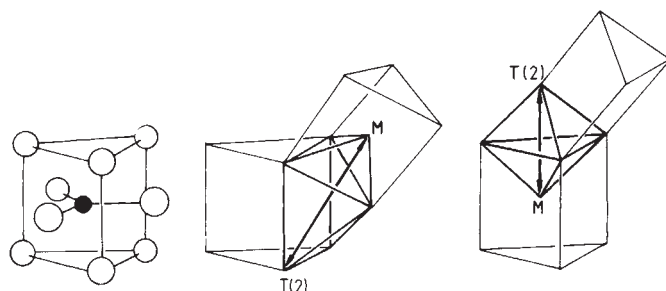


Fig. 1 Trigonal prismatic packing of transition metal atoms (large circles) around a metalloid atom observed in many crystalline alloys, together with the edge-sharing arrangement of trigonal prisms found in the Fe_3C structure (centre) and in Fe_3P (right). (Some atoms are omitted for clarity.) The two arrangements may be distinguished by distances from metalloids to second-neighbour transition metal atoms (M-T(2)). These can be visualized as the separation between two vertices of a tetrahedron sharing a face with a half octahedron (Fe_3C) and the distance between vertices of two tetrahedra sharing a triangular face (Ti_3P and Fe_3P), the latter generally being shorter as shown.

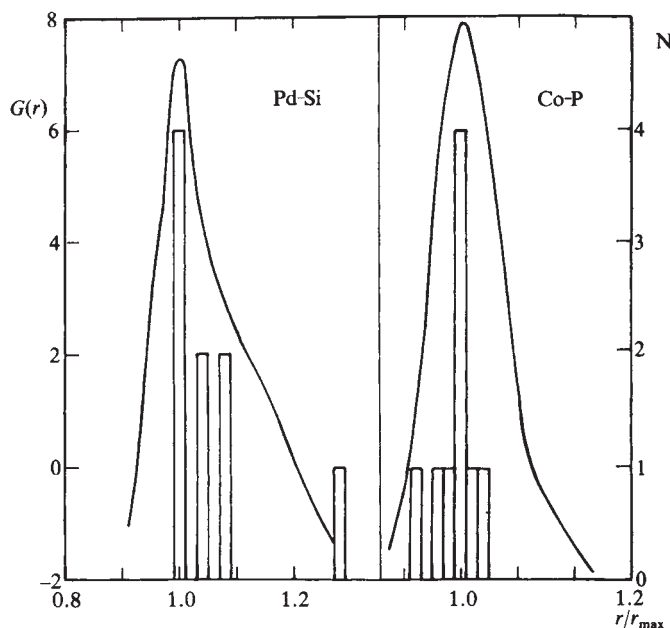


Fig. 2 Comparison of the profiles of the first-neighbour metalloid-transition metal distributions in crystalline and amorphous alloys. Bar charts represent the distribution of first neighbours (right-hand ordinate) in crystalline Pd_3Si (Fe_3C) and Fe_3P lattices, as a function of reduced interatomic distance, r/r_{max} , where r_{max} is the value corresponding to the maximum of the distribution. Curves represent Si-Pd and P-Co partial pair distribution functions (left-hand ordinate) for glassy $\text{Pd}_{84}\text{Si}_{16}$ and $\text{Co}_{81}\text{P}_{19}$ alloys^{5,6}.

is generally larger in Fe_3P and Ti_3P lattices causing extensive dilatation of the trigonal prism and distances to the nine first neighbours become almost identical (Fig. 2).

Extraction of T-M partial pair distribution functions for amorphous alloys requires an analysis of three essentially independent scattering measurements (for example, using X-ray and polarized neutrons) and this is rarely possible. Two good examples exist: $\text{Pd}_{84}\text{Si}_{16}$ (ref. 5) and $\text{Co}_{81}\text{P}_{19}$ (ref. 6). The former is potentially a 'Fe₃C glass' and the latter arguably related to Fe_3P on the basis of its radius ratio and crystallization products in Co-P-B ternary alloys. The marked differences between the profiles of the first Si-Pd and P-Co peaks is shown in Fig. 2. Furthermore, the T-M distribution for each glass is clearly similar to the bar chart for the corresponding crystalline phase. This strongly suggests that the environment of the non-metallic atoms is not random but is predominantly trigonal prismatic as in crystals. Moreover, distortions of the trigonal prisms resulting from the central metalloid atoms seem to be essentially identical in each type of amorphous and crystalline compound, suggesting a degree of stability for this type of coordination.

The disposition of trigonal prisms in the three crystalline lattices may be characterized as packing of trigonal prisms around half octahedra in Fe_3C or around tetrahedra in Ti_3P or Fe_3P lattices (Fig. 1) and it is possible to distinguish each type of packing through differences in the distances from a metalloid to second neighbour metal atoms. Second neighbour M-T(2) distances are shown in Fig. 1 and correspond to the vertex-vertex separation of a distorted tetrahedron sharing a triangular face with a half octahedron (Fe_3C) and the distance between vertices of two tetrahedra sharing a triangular face (Ti_3P and Fe_3P).

Second neighbour distances can be calculated for each pair of polyhedra as a function of the metalloid radius ratio, $p = r_{\text{M}}/r_{\text{T}}$. For the bi-tetrahedral configuration:

$$r_{\text{M-T}(2)} = (p^2 + 2p - \frac{1}{3})^{1/2} + (8/3)^{1/2}$$

and for the tetrahedral-octahedral configuration:

$$r_{\text{M-T}(2)} = [p^2 + 2p + 5 - 4(p^2 + 2p)^{1/2} \cos \beta]^{1/2}$$

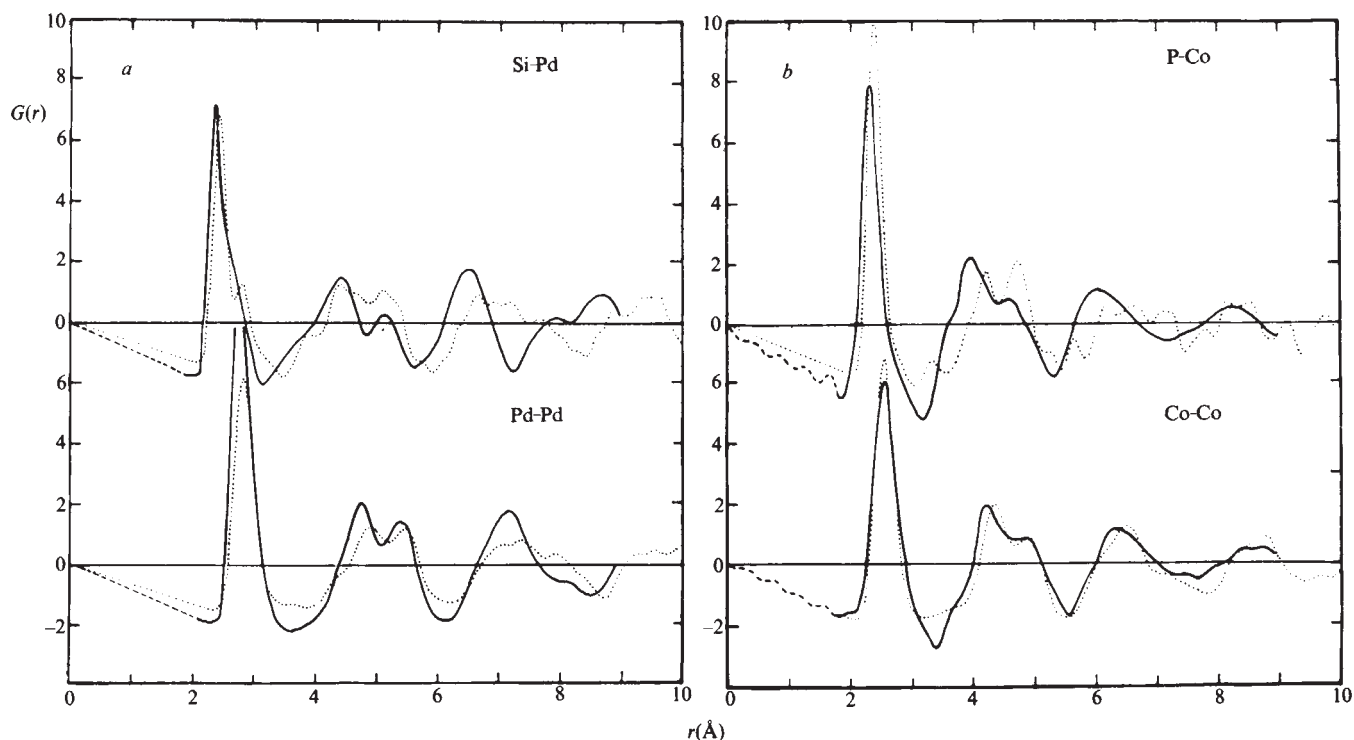


Fig. 3 Experimental (solid lines) and calculated (dotted lines) partial pair distribution functions for an amorphous Pd-Si alloy (a) and a Co-P alloy (b). Experimental data from Sadoc and Dixmier^{5,6}. Si-Pd correlation functions have been derived from two experimental measurements only: an approximation which should be adequate because Si-Si correlations are very weak and the form of this distribution can be assumed with sufficient accuracy.

where $\beta = \psi_1 + \psi_2$. The angles ψ_1 and ψ_2 are dihedral angles for a half octahedron and a distorted tetrahedron,

$$\psi_1 = \cos^{-1}(3^{-1/2}), \quad \psi_2 = \cos^{-1}[(3p^2 + 6p)^{-1/2}]$$

The radius ratio for Co-P is 0.83 (using Sadoc and Dixmier's values⁵) suggesting a second neighbour distance, $r_{M-T(2)} = 3.87$ Å for the bi-tetrahedral model which agrees well with the experimental value, 3.95 Å. The corresponding tetrahedral-octahedral distance is 4.14 Å. Conversely, for $\text{Pd}_{84}\text{Si}_{16}$ ($p = 0.74$) the best fit to experiment, $r_{M-T(2)} = 4.40$ Å, is given by the tetrahedral-octahedral model, 4.35 Å compared with 4.05 Å for the bi-tetrahedral arrangement. It seems, therefore, that the medium-range structure in these two amorphous alloys is essentially different and that there may be some equivalence of the medium-range topologies of crystalline and amorphous lattices.

This calculation may be confirmed by computer-simulation. The aperiodic model constructed with medium-range topology equivalent to Fe_3C (refs 1, 2) gives good agreement with the total neutron structure factor of amorphous Pd_4Si and the total radial distribution function. As Fig. 3a shows, this model also gives an excellent representation of the Pd-Pd and Si-Pd partial correlation functions obtained by Sadoc and Dixmier⁵. A split peak in the computed Si-Pd distribution is introduced by setting values for the equilibrium Si-Pd (vertex) distance of 2.4 Å, and 2.8 Å for the distance to capping atoms. This is a crude model of the nearest-neighbour arrangement shown in Figs 1 and 2. Both components of the second neighbour peak Si-Pd at 4.4 and 5.1 Å are satisfactorily reproduced, although the position of the first and the intensity of the second are somewhat inaccurate.

The model has been rescaled for Co-P without making any essential alterations to the topology, that is trigonal prisms and Fe_3C -type interconnections persist. Changes in nearest neighbour geometry have been introduced in an attempt to represent the local structure in Fe_3P -type alloys, specifically, the distinction between distances to vertex and capping atom mentioned above has been withdrawn. Computed Co-Co and P-Co pair correlation functions are shown in Fig. 3, together with measurements of Sadoc and Dixmier⁶. Co-Co distances are

again reproduced adequately to ~ 8 Å so that the total radial distribution function and structure factor, which are weighted by the Co-Co distribution, agree well with experiment⁷. The profile of the first P-Co peak and the position of the second component of the second neighbour peak at ~ 4.9 Å agrees with experiment, but the first component at 3.95 Å is inadequately represented by a peak at 4.17 Å in the computed data. Several attempts have been made, by altering values of the cohesive energy, changing equilibrium interatomic distances, to reduce this discrepancy and at the same time preserve a good fit to the rest of the data, but none has been successful. This difference, although small, seems to be significant and represents a closest P-Co(2) distance in an essentially tetrahedral-octahedral arrangement. Although a full model, built on packing principles exhibited by Fe_3P lattices, is required to prove the point, it seems that the bi-tetrahedral arrangement shown in Fig. 1 will be necessary to model successfully scattering data for cobalt phosphorus alloys.

Further experimental investigations of the partial correlation function of other amorphous alloys corresponding to Fe_3C and Fe_3P crystalline phases are needed and while the data, for which the errors have not been established, are sparse, current information suggests two conclusions. First, amorphous T-M glasses seem to contain a common local structural unit in which the detailed arrangement is basically determined by properties, such as the radius ratio, of the two elements. The local structure, therefore, is essentially identical to that of a compositionally-related crystalline phase. Second, medium-range structures for different combinations of elements are determined by rules, which are as yet incompletely deciphered, although the radius ratio again seems to be a dominant parameter. The result is that Fe_3C - and Fe_3P -type amorphous alloys (and crystals) possess essentially dissimilar medium-range topologies. This seems to be the first example of detectable medium-range structural differences in amorphous alloys.

Although there is little direct evidence, the composition-dependence of some magnetic properties of crystalline and amorphous Fe-(P, B) alloys^{8,9} indirectly supports the idea of differences in medium-range structures. For alloys with B:P ratio of ≤ 0.5 the stable crystalline form is Fe_3P , otherwise Ti_3P

(except for compositions close to Fe_3B where Ti_3P is metastable). The composition-dependence of the Curie temperature of both crystalline and amorphous alloys shows breaks corresponding to the transition between crystalline forms. These measurements have been interpreted in terms of changes in short-range order but the local structure is almost identical in the two crystalline forms. Therefore, even if changes of magnetic properties are directly related to local structure, these are probably accompanied and perhaps driven by changes in the mode of packing of prismatic units.

Recently, evidence from NMR measurements¹⁰ of the similarity in local structure in crystalline and amorphous alloys and of the dependence of local structure on composition has been reported. The environment of boron in glassy $\text{Ni}_{78}\text{P}_{14}\text{B}_8$ is found to be similar to that of Ni_3B which has the Fe_3C structure. In $\text{Mo}_{48}\text{Ru}_{32}\text{B}_{20}$, boron has cylindrical local symmetry which could reflect an essential similarity between boron coordination in the glass and in crystalline Mo_2B in which the atom is surrounded by eight Mo atoms at the corners of a square antiprism. However, as crystalline Fe_3P is also tetragonal, the NMR results are consistent with this type of structure also (Durand, personal communication).

Finally, local and medium-range topologies and the question of microcrystallinity are essentially distinct facets of any structural description of amorphous solids. The information presented here seems to be inconsistent with simple hard-sphere packing models for amorphous alloys; equally it is unnecessary to invoke any form of periodicity.

This work has been carried out during the tenure of an SRC Senior Fellowship. I thank the SRC and Pilkington Brothers Ltd for their support.

Received 16 October; accepted 26 November 1980.

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Formation and behaviour of coal free radicals in pyrolysis and liquefaction conditions

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In the past several years there has been a renewed interest in fundamental studies of coals and coal conversion. This interest is due to the expectation that an understanding of the structure of coal and of the fundamental processes during coal conversion would aid the development of technology for the utilization of coal on cost-effective and environmentally acceptable terms. In this context, electron spin resonance (ESR) is a promising technique, for it can be used to study paramagnetic centres in coal, including reactive free radicals which are generally assumed to have a key role in conversion processes. The ESR measurements of coals reported to date have all been measurements of pre-existing free radicals or free radicals remaining after some treatment. We report here the fabrication and utilization of a uniquely designed high-pressure-high-temperature ESR cavity and the first *in situ* observation of free radicals in coals.

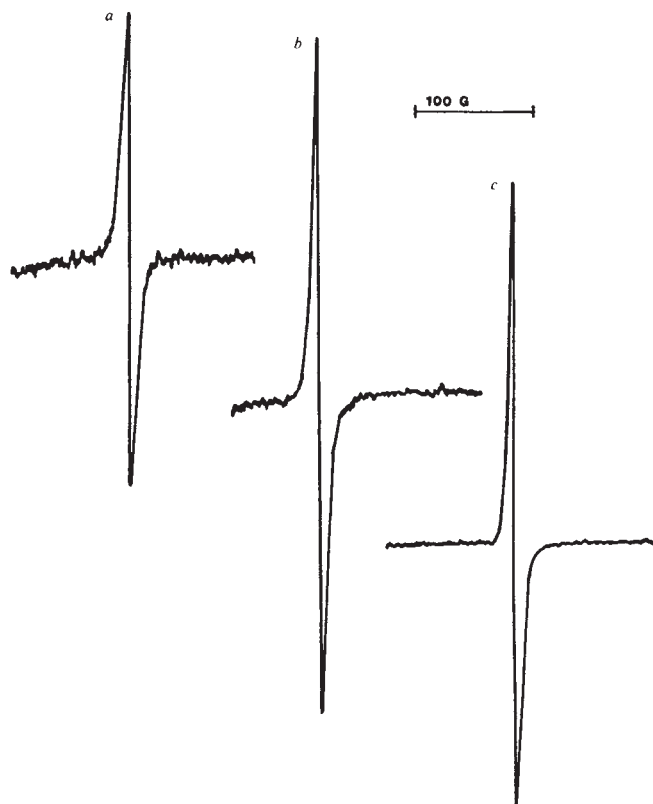


Fig. 1 ESR spectrum of Powhatan no. 5 bituminous coal under 10.335 MPa of hydrogen. Sample temperatures: a, 20 °C before heating; b, 450 °C; and c, 20 °C after 450 °C heating. Relative spectrometer gain factors are 8, 2.5 and 1, respectively.

This development makes it possible to perform a wide range of experiments such as coal conversion studies in process-relevant conditions, coke formation and various catalytic reactions. Findings from the *in situ* monitoring of the formation and behaviour of coal free radicals include: residual free radical concentrations following pyrolysis are similar to concentrations at reaction temperatures; manipulation of typical process variables effect a 12-fold increase in radical concentration, while the experimental error is much smaller ($\pm 40\%$); the determination that the most important process variable is temperature; process-derived solvents behave quite differently from single-component hydro-aromatic solvents such as tetralin.

Naturally occurring free radicals in coals were first reported in 1954. Subsequent studies have shown that the characteristics of pre-existing free radicals depend on the quality of the coals³, that they offer the possibility of probing the structure of coal^{3–5}, and that additional free radicals may be generated on various thermal treatments^{2,5–8}. This latter observation is particularly interesting, as it is generally assumed that stabilization of the free radicals through hydrogen abstraction from hydrogen-

Table 1 Comparison of yields in conversion of Powhatan no. 5 in ESR experiments

Solvent	Overall conversion (%)	Oil yields (%)
SRC-II*	27–36	2–6
Tetralin*	32–58	12–40

* 460 °C, low residence time.

donating solvents may be an important aspect of coal liquefaction⁹. Alternatively, recombination of free radicals at high temperatures could result in undesirable, high-molecular-weight species that cause reactor problems. Thus, it would be useful to be able to study the formation and/or reactions of free radicals *in situ*, that is in high-temperature and high-pressure conditions. The detailed design and operation of our ESR cavity that allows such measurements are discussed elsewhere¹⁰. A different high-pressure ESR application has also been described recently¹¹.

Figure 1 shows spectra that have been obtained from a Powhatan no. 5 HVA bituminous coal. This coal is one that has been used in pilot plant studies for the Solvent Refined Coal-II (SRC-II) process. Figure 1a is the first-derivative spectrum of the free radicals of natural coal at room temperature and 10.335 MPa pressure of hydrogen. Figure 1b is the signal obtained while the same sample is held at 450°C and the pressure of hydrogen in the sample is at 10.335 MPa. Finally, Fig. 1c shows the same sample after termination of the treatment and re-establishment of room temperature at hydrogen pressure of 10.335 MPa. By recording reference spectra, spin concentration and g-values can be readily determined, as well as how they may change with various treatments and time. On thermal treatment (450°C), the free radical concentration of Powhatan coal increases several-fold from 8.6 ± 1.0 to $59 \pm 5.0 \times 10^{18}$ spins per gramme. The observed increase is expected and it may be even greater for other types of coals and coal components⁵. On returning to room temperature, the observed residual free radical concentration is about the same ($53 \pm 5.0 \times 10^{18}$ per g) as had been observed at the high temperature.

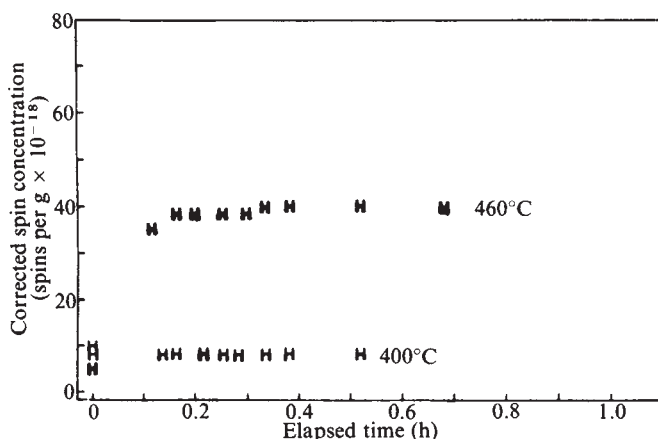


Fig. 2 Spin concentration plotted against elapsed time for Powhatan no. 5 and Tetralin. 1,200 p.s.i. H₂; HT = 3.

In addition to these pyrolysis experiments, more than 60 experiments, including a partially replicated 2⁵ factorial design, have been carried out to assess: (1) the role of liquefaction-process variables, such as solvent, temperature, gas, pressure and heating rate, on the formation of free radicals; and (2) how the degree of conversion and quality of product are correlated with the measured free radical parameters. ESR spectra can be obtained continually starting 3 min after the initiation of the heating cycle. Figures 2 and 3 show the variation of the free radical concentration as a function of residence time. These results allow the effects of temperature (400 and 460°C) to be assessed with two different solvents (tetralin and the heavy distillate of the SRC-II process-derived solvent from the Powhatan no. 5 coal). All other experimental conditions are identical in the four experiments shown (that is H₂ gas at 8.37 MPa and 3-min heating time to achieve reaction tempera-

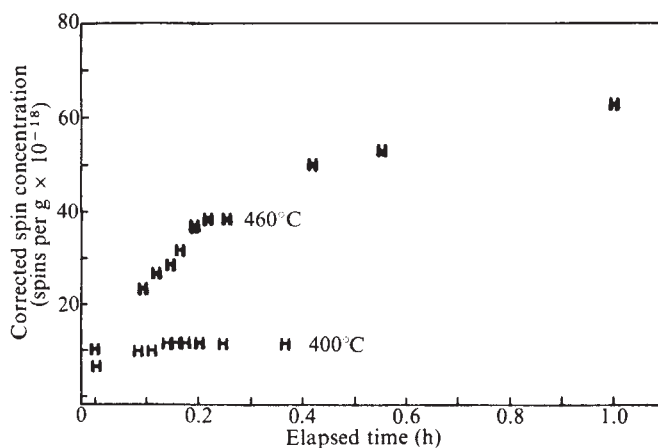


Fig. 3 Spin concentration plotted against elapsed time for Powhatan no. 5 and SRC-II HD. 1,200 p.s.i. H₂; HT = 3.

ture). With tetralin as a solvent (Fig. 2), the free radical concentration remains constant at 400°C as a function of residence time, while at 460°C it reaches its maximum value within 6 min. With SRC-II heavy distillate as solvent (Fig. 3) there is a small increase in free radical concentration at 400°C, while at 460°C the free radical concentration continues to increase even after 1 h of reaction time. The free radical concentration with tetralin as the solvent is lower than with the SRC-II heavy distillate. This is not unexpected, as tetralin is considered a good hydrogen donor and, therefore, can quench efficiently the coal free radicals.

We have also attempted to obtain data on the degree of conversion and quality of the product obtained. Such data are necessary to establish whether free radical chemistry determines the reaction paths in the liquefaction of coal⁹. The preliminary data available indicate that the overall tetrahydrofuran soluble conversion is lower when SRC-II distillate is the solvent (27–36% conversion) compared with the corresponding tetralin experiments (32–58%). For the same ESR experiments, the oil yields (pentane solubles) with SRC-II heavy distillate solvent are considerably lower (2–6%) compared with the corresponding tetralin runs (12–40%) (Table 1). These different degrees of conversion as well as the differences in the time-dependent spectra (Figs 2, 3) clearly indicate that some significantly different chemistry is taking place in the SRC-II process solvent experiments relative to the tetralin experiments. This is especially true in the first half-hour of the liquefaction reaction. The results also indicate very strongly the importance of both individual and combination effects of the process variables on the formation and behaviour of free radicals. Finally, the results shown here indicate the potential of using *in situ* measurements for understanding coal conversion processes on a molecular level, and thereby controlling the quality of the liquefaction product through the manipulation of the free radical chemistry by the various process variables.

This work has been performed partly under US Department of Energy contract 01-79ET14940.

Received 10 January; accepted 24 November 1980.

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Determination of geomagnetic archaeomagnitudes from clay pipes

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Archaeomagnitude determinations of a selection of clay pipes dateable to AD 1645±10 as well as studies of pottery samples from the same site and of the same age have been made. Values of the magnitude of the ancient magnetic field (B_{anc}), were obtained from two pottery sherds, two pipe bowls and three pipe stems. The values from the sherds and bowls agree within 2% and compare well with the average value of the magnitude of the magnetic field for the seventeenth century as determined by other archaeomagnetic studies. However, the pipe stems give values of B_{anc} which are significantly less than those from the bowls and pottery. We have not yet been able to explain this and thus we suggest that reliable archaeomagnitude determinations can be made from the bowls of clay pipes but not from the stems. Nevertheless, this result provides a new source of material for investigating variations in the geomagnetic field strength over the past 400 yr. Clay pipes have been manufactured in England since the end of the sixteenth century. In the firing process some pipes were broken and disposed of without ever having been smoked. One such collection, discovered at Rainford, Lancashire, in 1978, consisted of a series of discrete dumps including pipes, kiln debris and a small collection of contemporary used earthenware sherds. The internal consideration of the dumps suggested a very short period of activity and archaeologists (P. Davey, personal communication) ascribe all the material to the period 1645±10 yr. With such well-dated material, we set out to check whether or not reliable archaeomagnitudes could be obtained from the pipes.

The samples were all analysed using Shaw's¹ ARM technique. The maximum size of the samples used was determined by the sample holder which was designed to hold 1-inch diameter cylindrical cores. Figure 1 shows the graphs obtained for one of the pipe bowls. The points labelled R are rejected because they do not lie on a straight line of gradient = 1.00 on the plot of ARM 1 against ARM 2. The value of B_{anc} is determined from the slope of the normal remanent magnetism (NRM) against thermoremanent magnetism (TRM) graph using the equation

$$\frac{NRM}{TRM} = \frac{B_{anc}}{B_{lab}}$$

where B_{lab} is the laboratory magnetic field in which the TRM was acquired, in this case 50 μT.

All the samples were given their TRMs over a period of 7 h to reproduce as closely as possible the original slow cooling rate.

This minimizes the error introduced by different cooling rates for the NRM and TRM as described by Fox and Aitken². All the TRMs were installed using a field in the direction of the NRM to produce a TRM in the same direction as the original NRM within about 5°. This was to avoid the possible source of error caused by anisotropy³. This was very important as some of the samples were very anisotropic (see Table 1).

The results obtained for the pottery, bowl and stem samples are given in Table 1. To ascertain how close these results were to the actual value of the magnetic field at that time we averaged all of the known archaeomagnitude determinations for the seventeenth century as published by the Academy of Sciences (USSR)⁴. Seventy-two results for this period have an average value for B_{anc}/B_0 of 1.12 ± 0.13 where B_0 represents the present day field value at the place where the sample came from, and where the error is the r.m.s. deviation from the mean. For Rainford, therefore, we would expect the value of the ancient magnetic field strength to be 53.8 ± 6.2 μT. Table 1 shows that

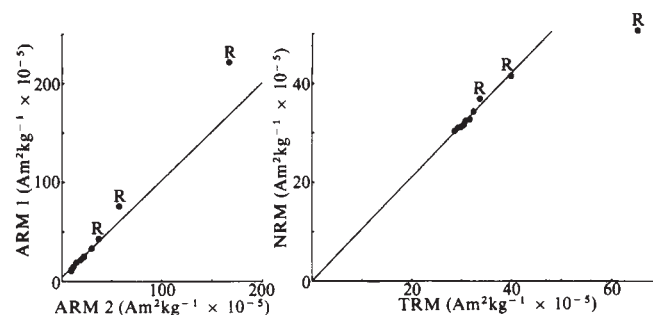


Fig. 1 Results for bowl sample R 7822. The graphs show all of the data for the NRM and TRM and ARM 1 versus ARM 2 plots. The points labelled R are rejected because they do not lie on the straight line of gradient = 1.00 drawn on the plot of ARM 1 versus ARM 2.

the values of B_{anc} from the pottery and bowl samples agree very well with each other and with the expected value. Averaging both sets of results we get 51.0 ± 2.0 μT for the bowls and 52.0 ± 1.0 μT for the pottery. However, the three pipe stems studied give widely ranging values which are all less than the expected value. Two of these stems, R7810 and R7813, give values which are of the order of 30% and 15% too low compared with the pottery and bowls, while the third stem, R7814, gives a value which is about 5% too low.

We have not yet succeeded in finding the reason for these low values from the stems, but we determined the degree of TRM anisotropy present in all of our samples, and these values are given in Table 1. Although two of the stems show the greatest degree of anisotropy, this in itself should not affect the value of B_{anc} as the TRM was always installed along the same direction as the NRM. Moreover, the third stem actually has quite a small degree of anisotropy.

These pipes were possibly placed in a radial pattern around a central pillar inside a cylindrical oven, with the bowls on the outside and the stems towards the centre. The lengths of the

Table 1 Magnetic field measurements of pottery bowl and stem samples

Sample	Type	NRM intensity ($A m^2 kg^{-1} \times 10^{-5}$)	TRM anisotropy (%)	B_{anc} (μT)	Average value of B_{anc} (μT)	Angle between NRM and long axis of stem
R 7811	Bowl	110	6	49 ± 2	51 ± 2	—
R 7822	Bowl	50	14	53 ± 3		—
R 7820	Pottery	14	6	51 ± 3		—
R 7823	Pottery	91	10	53 ± 3	52 ± 1	—
R 7810	Stem	65	34	36 ± 5		85°
R 7813	Stem	36	7	42 ± 1		25°
R 7814	Stem	134	26	48 ± 3	42 ± 5	10°

pipes may have been longer than the radius of the ovens, and so there would be a certain amount of stacking and overlapping of pipe stems in the central region. We calculated the effect of the distortion of the field produced by the overlapping stems, and concluded that it was too small to account for the low values of the field which we have obtained.

Another way in which the pipes could have been arranged was with the bowls at the outside of the oven, with the stems pointing radially inwards but also raised vertically so that the ends of the pipe stems met at some common point. As the inclination of the magnetic field is quite steep in England, this configuration would mean that the direction of the NRM in the pipe stems would in some cases be nearly parallel to the length of the stem and in other cases it would be transverse to the stem, whereas in the previously described configuration there is no obvious way that the NRM can be acquired along the length of the stem. When we analysed the NRM directions in the stems, we found that the direction of the NRM varied from angles of 10° to 85° with respect to the axis along the length of the stem (see Table 1). Furthermore, there was a correlation between this angle and the value of B_{anc} , but we cannot find any explanation for this.

We conclude, therefore, that the bowls of clay pipes may be used to determine archaeomagnitudes. As one of the major problems in archaeomagnetism is the availability of well-dated suitable material, any new source material such as these clay pipes is of great interest. Also, once the changes in the strength of the geomagnetic field over the past 400 yr have been satisfactorily determined, we may be able to date pipes of unknown age by this technique.

We thank P. Davey for samples and for his interest and help. K.P.G. is supported by NERC grant no. GR3/2396.

Received 24 September; accepted 10 November 1980.

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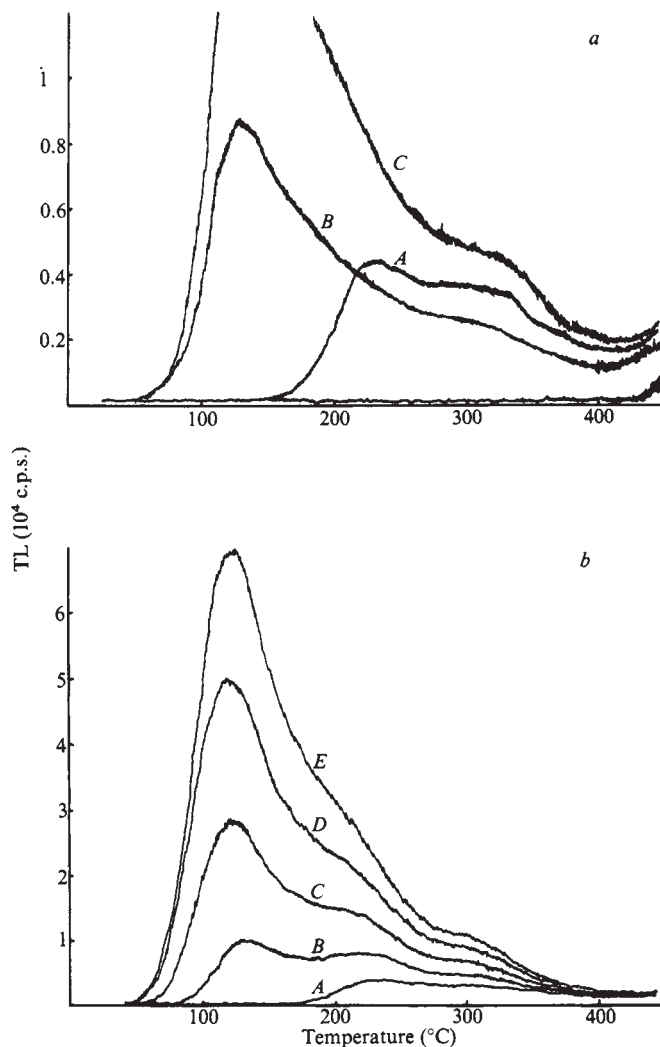


Fig. 1 TL glow curves for late Devensian loess. *a*: A, natural TL; B and C, TL due to 6.7 and 13.4 krad after heating. *b*: A, natural TL; B–E natural + 8.9, 13.4 and 17.9 krad respectively.

Thermoluminescence dating of late Devensian loesses in southern England

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Scattered across southern England are many isolated deposits of loess-like material¹. A few, such as that at Pegwell Bay in Kent, are highly calcareous and unweathered but most have been reworked by fluvial or colluvial processes. There is good stratigraphical evidence for a few pre-Devensian loesses, also in Kent, but dating of more recent loess has so far been based on indirect evidence. Much work has been done on the Pegwell Bay loess as it is the most extensive, truly aeolian loessic deposit in Britain. Kerney² compared the late Devensian deposits in the Isle of Thanet and at Pegwell Bay with similar deposits in Holland and Belgium where radiocarbon dates have been obtained for interstadial deposits. Correlation of the East Kent deposits with these in Northern Europe indicates that the loesses in Kent were formed between 30,000 and 14,000 yr ago. I report here dates for six of the more recent deposits in southern Britain from the Scilly Isles to Kent. The dates have been obtained on the loess itself, using a recently developed thermoluminescence (TL) dating technique^{3,4}, and confirm the ages as being late Devensian.

The TL dating of any sedimentary deposit, whether marine or terrestrial, is based on the assumption that exposure to sunlight during the weathering and transport of the detrital grains is sufficient to remove most of their previously acquired TL signal. The TL signal in a mineral is due to the untrapping of electrical charges in the crystal when it is heated, the trapped charges having been produced by ionization due to the decay of natural radioactive elements in the sediment. The most common TL-sensitive minerals are quartz and feldspar and these are the dominant minerals present in loess. In southern England the peak grain size of the deposits decreases from $\sim 45 \mu\text{m}$ in Kent to $25 \mu\text{m}$ in the south-west¹ suggesting that the loesses, if contemporary, have been derived from a source in the North Sea. In this study polymineral grains in the range 4–11 μm were used, 0.5-mg samples being deposited onto 1-cm diameter aluminium disks for TL measurements. The disk is heated in an oxygen-free atmosphere from room temperature to 450°C at 2°C s^{-1} and the resulting TL output recorded as a function of temperature known as a glow curve. The glow curves for all six samples are very similar to each other and to those obtained for detrital grains from ocean sediments³.

Only the Pegwell Bay sample 1a could be described as pure aeolian loess. The St Agnes sample 1d looked pure but did not

Table 1 Thermoluminescence data and radioactivity analyses

Sample QTL no.	ED + I (krad)	% K ₂ O	Total α -count rate (ks ⁻¹ cm ⁻²)	Th* (p.p.m.)	U* (p.p.m.)	<i>a</i> (± 0.05)	Δ_{sat}	Dose rate (rad yr ⁻¹)	TL age (kyr)
1a†	5.0 ± 0.0 = 5.0 ± 0.4	1.77 ± 0.02	0.669 ± 0.013	9.7 ± 0.8	2.6 ± 0.2	0.084	0.50	0.337	14.8
1b	5.8 ± 0.6 = 6.4 ± 0.5	1.90 ± 0.01	0.992 ± 0.012	16.0 ± 1.5	3.0 ± 0.4	0.092	0.49	0.441	14.5
1c	5.9 ± 0.3 = 6.2 ± 0.4	1.81 ± 0.01	0.665 ± 0.013	8.8 ± 1.2	3.0 ± 0.3	0.075	0.48	0.330	18.8
1d	6.4 ± 0.4 = 6.8 ± 0.5	2.23 ± 0.04	0.653 ± 0.006	9.7 ± 0.9	2.6 ± 0.3	0.085	0.26	0.365	18.6
1e	6.1 ± 0.0 = 6.1 ± 0.4	1.67 ± 0.00	0.835 ± 0.013	9.8 ± 0.8	4.1 ± 0.2	0.089	0.48	0.383	15.9
1f	6.2 ± 0.0 = 6.2 ± 0.4	1.71 ± 0.02	0.670 ± 0.011	7.6 ± 0.9	3.4 ± 0.3	0.085	0.35	0.333	18.6

* Calculated assuming equilibrium of the ²³⁸U and ²³²Th decay chains.

† Repeat measurements on three subsamples of 1a gave the same age within errors.

break up when placed in water. The other Scilly Isles sample, from St Mary's, (1f) had a similar texture but had large brown stains running through it. The sample from Barton in the New Forest area was also uniform but contained fine roots. Sample 1b from Sturt Pond in the New Forest area and 1e from the Lizard Peninsula, Cornwall, also contained roots and many small pockets of darker soil which are likely to have been washed in from above. The last two samples were initially considered to be unsuitable for TL dating because of likely contamination but were included for completeness.

Figure 1a shows typical glow curves for the Barton sample, showing natural TL and response to subsequent laboratory irradiations. To obtain the age of the sediment since its last exposure to sunlight, the natural TL signal needs to be compared with that induced in it by a known radiation dose. A ⁹⁰Sr/⁹⁰Y β source was used with method (c) of Wintle and Huntley⁴ to separate the TL due to radiation exposure since burial from that which was not bleached by the original sunlight exposure (termed the residual component). Figure 1b shows the effect of additional doses of radiation on top of the natural TL for the same sample (on a different vertical scale): the lower temperature peaks are the more sensitive. Above 350 °C the residual component is responsible for >50% of the natural TL and this, combined with the low TL sensitivity in this region, makes it impossible to obtain the equivalent radiation dose (ED) in this region. The values of the ED obtained in the glow curve temperature region 260–320 °C are given in Table 1. In this region the value of the ED obtained does not vary with glow curve temperature. Integrating over this temperature range, the residual signal is equivalent to 40% of the total natural TL for samples 1b to 1f and 50% for 1a; ignoring it would have added about 3.5 krad to the ED of each sample. This is typical of residual signal obtained by the same technique for polymineral samples of ocean sediments having EDs in the range 3–26 krad (ref. 3). The TL signal from samples which have been irradiated after heating indicates that the TL at lower doses is not perfectly linear and an intercept correction (*I*) is made as in the case of the quartz inclusion TL dating method for pottery⁵. The error in the determination of the past radiation dose (ED + *I*) is $\sim \pm 7\%$ and is due mainly to the subtraction of the residual signal.

To obtain the age of the sediment, the radiation dose obtained from the TL measurements must be combined with the annual dose rate obtained from radioactivity analyses. The contribution from ⁴⁰K is calculated from the total K₂O content measured by atomic absorption spectrometry and for these samples the ionization due to ⁴⁰K is responsible for about a third of the natural TL. Apart from a 14 mrad yr⁻¹ contribution due to cosmic radiation, the remaining radiation is due to the α , β and γ radiation from the decay of ²³⁸U and ²³²Th chains present in the sediment. Their activity is measured by a ZnS α scintillation counting technique⁶. Table 1 shows that the total α count rate is

very similar for the four cleanest samples. The two which were observed to contain small pockets of darker soil had larger α counts, perhaps indicating recent contamination. (Sub-sampling of these specimens also gave a scatter of 10% whereas the cleaner samples were reproducible to within 5%. Clean water was dripped continuously through samples 1a and 1b for 10 days after measurement and on recounting no change was observed for 1a but a loss of 25% in total α count was observed for 1b.) If the U and Th decay chain contributions had been the same as for the other samples, the ages for 1b and 1e would have been 17,200 and 18,200 yr respectively.

The α efficiency factor, *a*, was measured for each sample and the annual dose rate calculated using the energy conversion factors of Bell⁷ and assuming a mean water content of 20%. The saturation water content was measured for each sample in the laboratory. However, except for the two samples from the Scilly Isles, 1d and 1f, the samples formed a slurry and it was thought incorrect to try and estimate a mean annual water content from these values. The 20% value was found to be the mean *in situ* value for British brick-earth deposits. The water content is important because of the absorption of energy by the water which would otherwise be absorbed in the minerals to produce a higher TL signal. The age calculated is directly dependent on the per cent water content and this is the greatest source of error in dating these samples. Anomalous fading tests were carried out and no losses within the experimental reproducibility limit of $\pm 4\%$ were found in the 260–320 °C temperature range.

This is the first application of TL dating to late Devensian loess, although apparently successful TL dating of the quartz fraction of older loess has been reported in the Soviet Union⁸. The uncertainty in estimating the water content and the residual signal are the two major sources of error in the age and an overall error of $\pm 20\%$ should be applied to these dates; this makes it impossible to separate the six samples studied here but they are easily distinguished from older loesses which I am studying. TL dating can thus resolve the controversy concerning the presence of late and/or early Devensian loess in Britain.

I thank Dr J. A. Catt for samples and for stimulating discussion, Mrs M. Miller for potassium analyses and Dr M. J. Aitken for use of a ²⁴²Cm α source. Financial support was provided by a NERC grant to Dr N. J. Shackleton.

Received 29 August; accepted 15 December 1980.

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Bermuda sea level during the last interglacial

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Bermuda is a stable, mid-oceanic carbonate platform for which a particularly complete record of sea level fluctuation during the period ~135–70 kyr has been reconstructed from $^{230}\text{Th}/^{234}\text{U}$ dating of corals and speleothems, amino acid racemization dating of molluscs, and a reinterpretation of geological field relations. During the past 5 yr Bermuda has been the focus of an extensive geochronological programme which has included a detailed re-examination of the geology (to be reported elsewhere^{1–3}). Here we focus only on the latest interglacial sequence. We use the stratigraphic nomenclature of Land *et al.*⁴, but will present new age data and interpretations of geological relationships. This information, as it relates to the last interglacial period, is given in Tables 1 and 2 and summarized in Fig. 1. We show that during the last interglacial period, sea level was only above its present level for a few thousand years at about 125 kyr when it stood at +4 to +6 m. At ~105 and ~85 kyr, sea level rose to between –15 and –20 m as constrained by ages for submerged speleothems and eolianites. At ~95 kyr sea level in Bermuda was at least –15 m below present level.

The Bermudas are a group of tectonically stable, Pleistocene-age islands in the mid-Atlantic (35°N, 65°W) consisting of intercalated and interfingering shallow-water marine calcarenites, eolianites and terra-rose palaeosols deposited on a stable volcanic pedestal. At least four complex, superimposed suites of marine and eolian carbonates occur in a general pattern of seaward accretion with the younger units lying closer to the coast. Within each suite there is an alternation of carbonate facies, with successive suites generally separated by palaeosol horizons. Caves formed by ground-water solution and collapse are common within the older, more lithified carbonate units and often extend below present sea level and are partially or totally filled with seawater. Extensive deposition of calcium carbonate speleothems (stalactites, stalagmites and flowstones) at elevations above and below present sea level is characteristic of the older caves.

Bretz⁵ and Land *et al.*⁴ established the conceptual framework within which Bermudian geology must be considered. They

recognized that the carbonate formations are not simple stratigraphic units arranged in layer-cake fashion which can be separated solely on the basis of lithological or palaeontological criteria, and pointed out that an understanding of depositional mechanism is critical to the interpretation of Bermudian geology. Eolianites comprise over 90% of the exposed carbonate rock in Bermuda and represent lithified, transverse-lobate dune ridges⁶. These can be observed in some cases to merge laterally into marine carbonates and therefore must have been deposited during times of interglacial high sea stand. Thus, preserved littoral marine carbonates and patch reefs must have formed during interglacial periods at times when sea level was at or above its present level and eolianites can only have formed when the Bermuda platform (~20 m) was submerged to provide a source of sand for eolian shoreline dunes, but with sea level not necessarily as high as at present. During intervening glacial periods of depressed sea level, the Bermuda platform was subaerially exposed, and accumulation of terra-rose or regolith palaeosols and deposition of speleothems in caves at elevations below present sea level occurred coincident with vadose carbonate diagenesis. Thus, because the Bermuda platform is in an area of the world not significantly affected by either the effects of vertical displacement due to tectonic uplift or isostatic deformation through ice or water loading^{7,8}, Bermudian geology represents an approximate palaeosea level gauge, presumably recording absolute eustatic fluctuation of late Pleistocene sea level.

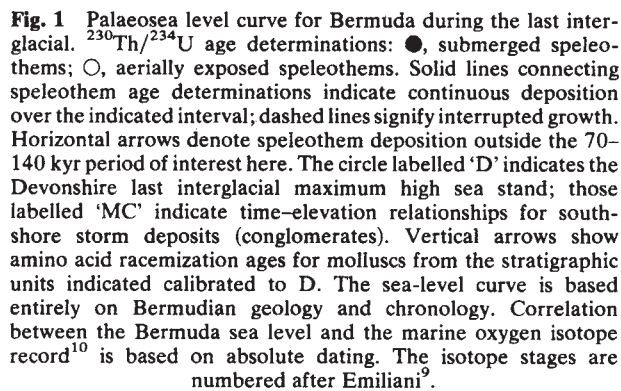
The last interglacial period in the marine record from ~135,000 to 75,000 yr BP is commonly assigned to Stage 5 of the marine oxygen isotopic record^{9,10}. As Fig. 1 shows, the Bermuda platform was probably submerged for most of this time. Before Stage 5, during Shore Hills time, sea level was below the edge of the Bermuda platform, then rose rapidly, passing –13 m at ~134 kyr, to the Devonshire +4 to +6 m high stand at 125 kyr. Here it produced a wave-cut notch in the older Belmont Formation, deposited the Devonshire marine calcarenites and flooded what are at present the air-filled portions of many caves resulting in the development of aragonitic surface coatings on actively forming stalagmites and stalactites up to elevations of +3 to +4 m. Sixteen $^{230}\text{Th}/^{234}\text{U}$ dates on Devonshire corals from sites around Bermuda where stratigraphic relationships are clear range from 134 ± 8 to 118 ± 6 kyr with a mean of 125 ± 4 kyr (Table 2), thus correlating the Devonshire Formation with the Rendezvous Hill terrace in Barbados^{11,12}, reef complex VII in New Guinea¹³ and the Wiamanalo shoreline in Hawaii¹⁴. Sea level stood at this level for only a short period before quickly falling off the Bermuda platform as indicated by initiation of speleothem deposition at elevations below present sea level by ~120 kyr. This regression is reflected in the geological record by the development of the Harrington Formation which gives both U-series and amino acid racemization ages of ~120 kyr (Table 2).

The remainder of Stage 5 time in Bermuda is a long and complex episode of dune building and soil deposition resulting from periodic submergence and exposure of the Bermuda platform but with sea levels always below present level. The amino acid racemization ages of ~105 and ~86 kyr for two post-Devonshire eolianites (Table 2) record the timing of these transgressions. The dates correlate well with isotope Stages 5c

Table 1 New $^{230}\text{Th}/^{234}\text{U}$ ages for Bermuda stalagmites

Sample no.	Depth (m)	U conc. (p.p.m.)	($^{234}\text{U}/^{238}\text{U}$)	($^{230}\text{Th}/^{234}\text{Th}$)	($^{230}\text{Th}/^{232}\text{Th}$)	Age (kyr BP)
75008:02(t)	–3	0.08	1.04 ± 0.04	0.47 ± 0.03		68 ± 6
:01(b)	–3	0.07	1.11 ± 0.03	0.61 ± 0.03	>200	97 ± 9
77520:01(t)	–15	0.14	1.17 ± 0.03	0.09 ± 0.02	30	10 ± 2
:03(m)	–15	0.09	1.05 ± 0.04	0.30 ± 0.05	>200	39 ± 7
:02(b)	–15	0.08	1.60 ± 0.02	0.67 ± 0.03	>200	111 ± 9
77522:01(t)	–13	0.08	1.53 ± 0.02	0.75 ± 0.03	110	134 ± 11

t, top; m, middle; b, base.



The two most persistent problems of Bermudian geology have been the patchy marine conglomerates which occur along the south shore at elevations between sea level and +11 m, and a complex of evenly bedded, seaward-dipping sands which are present along the north shore at elevations up to about +20 m. Land *et al.*⁴ assigned the conglomerates to one lithological

The north-shore sands we now assign to the Devonshire Formation on the basis of amino acid racemization ages which are the first absolute age data for these deposits. The sea stand responsible for the deposition of the north-shore sands, 'perhaps as high as 20 m (Blackwatch Pass)'¹⁴, has always been based on interpretation of depositional environment of the uniform seaward-dipping section. Some geologists might claim that all the units are marine, others have argued that they are all eolian

Well developed red palaeosol of island-wide extent with abundant palmetto stump casts.

‡ Data from ref. 1.

Table 3 Comparison of sea level elevations during the last interglacial period

Locality	Time (kyr)	Sea level (m)	Ref.
Bermuda	125	+4 to +6	16, this study
	118	> -20	
	105	-15 to -20	
	95	> -20	
	85	-15 to -20	
New Guinea	120	+8	13
	110	-40	
	105	-14	
	82	-14	
Barbados	125	+5	11
	105	-43 ± 20	
	82	-42 ± 20	
Marine oxygen isotope record*	125	+11	10
	116	-19	
	110	-	
	105	-17	
	95	-18	
	84	-11	

* For core V28-238 assuming -0.1‰ change in $\delta^{18}\text{O}$ of foraminifera calcite per 10 m rise in sea level.

backset beds³. We have found no definite evidence but have recognized apparent beach cusps as high as +3 m above present sea level, and conclude that higher beds must be due to reworking during storms (hurricanes) during Devonshire time. The underlying eolianites, which give an amino acid age of ~140 kyr, thus represent an early phase of the Devonshire transgression, during which time the extensive North Lagoon could have supplied sand and stone⁵.

Because the Bermudian palaeosea level record for the last interglacial presented here (Fig. 1) is particularly complete, it has important implications for the idea of glacial surge. Hollin¹⁵ has recently argued that a catastrophic surge of the East Antarctic ice sheet caused a rise in worldwide sea level to about +16 m at ~95 kyr. Although such an event should have left some evidence of its occurrence in the geological record of island and coastal areas, it can be argued that it might not be reflected in either the marine oxygen isotope or tectonic island sea level records because both of these are relatively low-resolution measures of global ice volume integrated over a time scale of a few thousand years. Hollin¹⁵ recognized that his hypothesis must be tested in an area where a highly resolved sea level record for the last interglacial is preserved. Bermuda is one such locality, and thus the palaeosea level record presented here is a crucial test for such an ice surge.

Although Bermuda is cited by Hollin¹⁵ to support a +16-m high sea stand at ~95 kyr, the reappraisal of geological relationships resulting from our chronological and field studies has revised the tenuous correlation on which a possible +20 m post-Devonshire high sea stand was based. Figure 1 shows that sea level in Bermuda was only once above its present level during Stage 5; that is during Devonshire time at 125 kyr when it stood at +4 to +6 m. Otherwise, from ~135 to 70 kyr sea level was below ~-15 m. There is no geological evidence to the contrary. There is no indication of a former freshwater table at elevations above present sea level, except the +2 m feature relating to the penultimate high sea stand which deposited the Belmont Formation between ~230 and 200 kyr (ref. 1). Neither have we observed any overgrowths nor surface coatings of marine aragonite and serpulid worm shells on any speleothems above +3 to +4 m in the more than 20 caves investigated nor found any interior aragonite layers in speleothems younger than 120 kyr. Such features are present to some extent on the surface of all speleothems presently submerged in seawater.

In Crystal Cave, stalactites at +2 to +4 m above sea level typically contain one interior layer of such marine deposition which can be easily observed because the lower portions of these speleothems have been broken by human activity to reveal a cross-section through these deposits. For one such stalactite previously studied, the aragonitic layer only extended upwards inside the stalactite to an elevation of +3 to +4 m, above which calcite crystal growth was continuous across the axis of the entire stalactite. $^{230}\text{Th}/^{234}\text{U}$ dating gave an age of 130 ± 10 kyr for the calcite layer just inside the marine layer and 110 ± 14 kyr for the outer calcite layer thus independently documenting the absolute elevation of the Devonshire high sea stand at 125 kyr (Fig. 1) (ref. 16). Certainly one would expect to observe two such sediment layers in these stalactites and marine overgrowths and surface coatings on subaerial travertines and flowstones as well as evidence for an elevated freshwater table between +4 and +16 m if there had been a post-Devonshire sea level rise to +16 m. Also, neither the extensive Devonshire marine calcarenites and relatively un lithified Pembroke or Southampton eolianites, nor the wholly unconsolidated, Harrington Formation have been reworked to any extent. All these units are exposed along the south shore of Bermuda at or near sea level, not more than 1–2 km from the platform edge, at present a very dynamic, high-energy environment. It, therefore, seems most unlikely that a +16 m rise in sea level over only a few hundred or thousand years, as required by the surge hypothesis¹⁵, could have happened without some reworking, eroding, and re-depositing of these units. The discontinuous marine conglomerates cannot be surge-derived unless the coincidence of their ages with the well recognized isotope Stage 5e, 5c and 5a high sea stands is fortuitous, with surge events occurring within each of these transgressions, and demonstration that deposition of stalagmites below present sea level could have been interrupted by a marine transgression and then resumed without leaving behind physical or petrographical evidence to this effect.

Finally, the Bermuda palaeosea level record presented here is in basic agreement with both the marine oxygen isotope¹⁰ as well as the New Guinea¹³ and Barbados^{11,12} sea level records (Table 3). Although there are some internal inconsistencies among these various ice-volume records which reflect both geological and chronological uncertainties, the overall agreement is striking. However, although casting serious doubt on Hollin's¹⁵ postulated surge of the East Antarctic ice sheet at 95 kyr, these results do not question either the theory or reality of the general concept as discussed by Aharon *et al.*¹⁷. The Devonshire high sea stand may in fact represent a surge event at 125 kyr superimposed on the general submergence of the Bermuda platform during the last interglacial period.

The $^{230}\text{Th}/^{234}\text{U}$ coral and speleothem dating programme and palaeosea level work in Bermuda has been supported by a grant from the NSF (EAR 77-23026) and a Wilkinson Fellowship from the Bermuda Biological Station. The manuscript was typed by Miss J. McLean and illustrations drawn by J. Bennett.

Received 3 July; accepted 18 November, 1980.

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Low $^{15}\text{N}/^{14}\text{N}$ in hydrothermal vent animals: ecological implications

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Low-trophic-level animal biomass from the Galápagos and 21°N hydrothermal vents was found to have $^{15}\text{N}/^{14}\text{N}$ ratios which are lower than those previously reported for organic nitrogen¹⁻⁶, ammonium^{1,2,5}, and nitrate^{1,2,7,8} in deep Pacific Ocean water. These nitrogen isotope ratios of vent animals are, however, near those of deep-oceanic N_2 (refs 1, 2, 7, 9) and resemble those of marine biota associated with N_2 fixation^{2,10}. These observations suggest that organic nitrogen of nutritional importance to vent animals is initially synthesized within the vent environment and this synthesis may be preceded by N_2 fixation.

It has been argued¹¹⁻¹⁹ that local bacterial chemo-autotrophy is the primary source of biomass and energy for the dense invertebrate communities found at Pacific Ocean hydrothermal vents. Part of the evidence for vent primary production is the difference between the $^{13}\text{C}/^{12}\text{C}$ of vent animals and the $^{13}\text{C}/^{12}\text{C}$ of animals in the overlying pelagic environment^{13,19}. It was reasoned that measurement of vent animal $^{15}\text{N}/^{14}\text{N}$ would provide further clues as to the base and the structure of these vent food webs.

A variety of animal tissues from hydrothermal vents were analysed including: trophosome and vestimentum from *Riftia pachyptila* Jones (a vestimentiferan worm), mantle tissue from *Calypotegna magnifica* Boss and Turner (vesicomyid clams), and crab muscle from *Bythograea thermydron* Williams (brachyuran crabs). These animals had been originally taken from either the Galápagos or the 21°N hydrothermal vent sites through the submersible Alvin, as indicated in Fig. 1. These tissues were dried at 60°C and ground, and the nitrogen present in 5–10-mg replicate subsamples of these tissues was completely converted to N_2 using methods described by Stump and Frazer²⁰. The $^{15}\text{N}/^{14}\text{N}$ relative to air (Fig. 1 legend) of these N_2 gas samples was determined by David Winter using a Varian MAT 250 ratio mass spectrometer.

Figure 1 shows vent animal $\delta^{15}\text{N}$ ranges from +1.8 to +9.8‰, with the values generally increasing as a function of the animal's assumed trophic level. This animal $\delta^{15}\text{N}$ increase with trophic status corroborates observations^{2,4,10,21-25} that food chain/heterotrophic processes tend to increase ^{15}N relative abundance in the resultant animal biomass. Consequently, if vent animals were utilizing sedimentary organic nitrogen that had been produced and biologically processed on its journey through the more than 2 km of water overlying the vents, one would expect the $\delta^{15}\text{N}$ of vent animals: first, to exceed the $\delta^{15}\text{N}$ of plankton encountered in the surface layers of the Pacific Ocean, commonly 5–10‰ (refs 1, 2, 5, 10, 26), and second, to equal, if not exceed, the $\delta^{15}\text{N}$ of deep-ocean sedimentary organic nitrogen, 5–13‰ (refs 1–6). The relatively low $\delta^{15}\text{N}$ of assumed primary producer-consumer tissues from the vents, 1.8–4.9‰ (Fig. 1), however, suggests that the organic nitrogen present in these tissues has undergone relatively little biological cycling and is, therefore, of local, rather than pelagic-sedimentary origin.

Additionally, the striking similarity between the $\delta^{15}\text{N}$ of low-trophic level vent animal tissues and the $\delta^{15}\text{N}$ of marine organisms associated with N_2 fixation (Fig. 1) suggests that the production of the organic nitrogen found in vent animals is preceded by N_2 fixation. Both the concentration and the low $\delta^{15}\text{N}$ of dissolved N_2 are relatively constant throughout the ocean^{1,7,9}, and little nitrogen isotopic fractionation is known to occur during fixation of N_2 and subsequent organic nitrogen synthesis^{2,10,27-31}. The yet-to-be measured $\delta^{15}\text{N}$ of the NH_4^+ and the NO_3^- present³² in the vicinity of the vents may, however, prove these ions as likely as N_2 to be precursors to vent animal nitrogen. Large nitrogen isotope fractionations incurred during nitrate or ammonium assimilation and biomass production by some microbes and by some autotrophs in certain conditions^{2,28,31,33-35} may also be factors causing vent organisms to be relatively depleted in ^{15}N .

Whatever the synthesis pathways and their inorganic starting compounds, the existing isotopic evidence indicates that the organic nitrogen as well as the organic carbon^{13,19} found in vent animal biomass are largely produced by non-pelagic and non-photosynthetic processes.

This research was made possible through the generosity of Mr Bud Smithey and Drs Robert Hessler, George Somero, Horst Felbeck, James Childress and Ian Kaplan. Support was provided in part by a joint US Bureau of Land Management/Department of Energy contract No. EY-76-3-03-0034

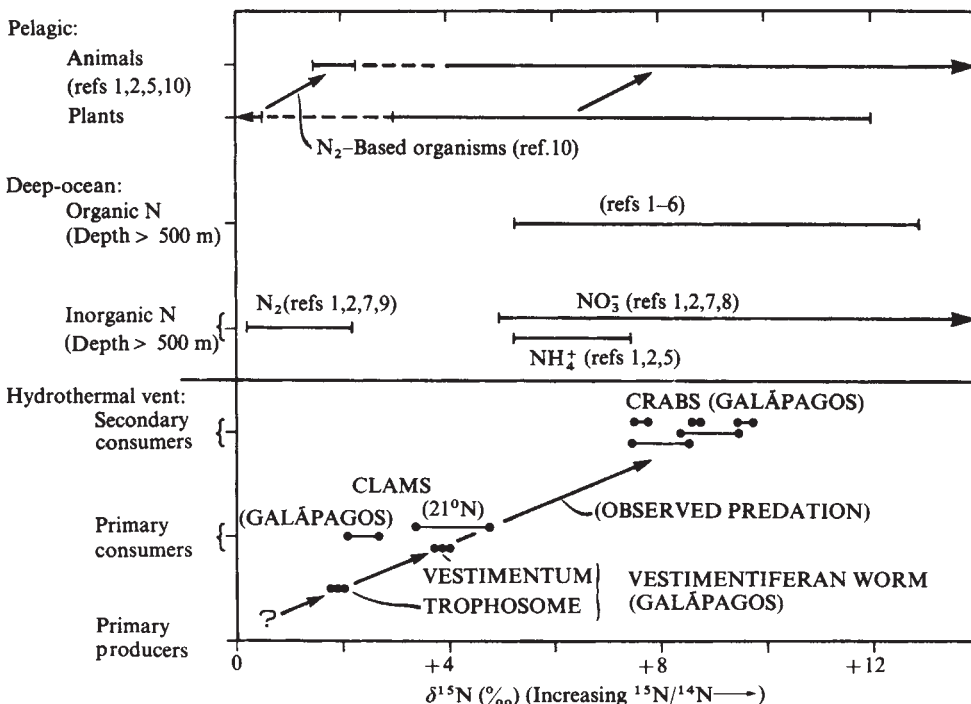


Fig. 1 The $\delta^{15}\text{N}$ of various Pacific Ocean nitrogen pools where:

$$\delta^{15}\text{N} = \left\{ \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{air}}} \right] - 1 \right\} \times 10^3 \text{‰}$$

The vent sites, Galápagos (0.8°N, 86.1°W) and 21°N (20.9°N, 109.1°W), are located at ocean depths of 2.5 and 2.6 km, respectively. The assumed trophic levels and transfers (diagonal arrows) of hydrothermal vent animals/tissues are based on knowledge of related species, and on observations by Drs R. R. Hessler, G. N. Somero, H. Felbeck and J. J. Childress. The trophosome of the vestimentiferan worm is at least partly, if not largely, composed of autotrophic tissues¹⁶⁻¹⁸. Replicate analyses of a single individual's tissue are connected by a horizontal line.

with UCLA, as well as NSF grants OCE-78-08852 (to Chidress), OCE 78-08853 (to Somero), and OCE 78-10460 (to Hessler). This is contribution no. 23 of the Galápagos Rift Biology Expedition supported by the NSF.

Received 11 August; accepted 7 November 1980.

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Fourier analysis of test shape of planktonic foraminifera

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The exact characterization of test shape is important in morphological studies of foraminifera and other marine microfossils. Most biometric studies of microfossils, however, have only measured test diameter or other linear components¹. The tests of planktonic foraminifera exhibit a wide range of morphological traits, some of which are related to environmental conditions and others to the phylogenetic history of the taxon^{1,2}. Mean test size or linear measurements of chambers and/or whole tests of fossil foraminifera can be used for palaeoecological interpretations from the morphological variations^{3–10}. We report here that Fourier shape analysis could be an objective and unambiguous biometric method for determining the components which comprise the shape of foraminifera. Fourier shape analysis has been successfully applied to fossil ostracods^{11,12}, fossil bryozoans^{13,14} and miospores¹⁵. In the present study, Fourier series shape analysis was performed on the planktonic foraminifera, *Globorotalia truncatulinoides*, from the southern Indian Ocean, to differentiate palaeo-environmentally significant trends in the test shape of this species. Kennett¹⁶ and Takayanagi *et al.*¹⁷ have used linear test measurements to describe latitudinal gradients in the test morphology of *G. truncatulinoides*. Highly conical forms dominate in tropical waters, whereas more compressed, bi-convex forms dominate in cool water.

Table 1 Core top locations used in the Fourier analysis of *Globorotalia truncatulinoides*

Core	Lat (°S)	Long (°E)
V20-175	22° 18'	68° 00'
RC11-145	25° 29'	110° 01'
E48-11A	29° 40'	97° 32'
E48-46A	31° 09'	83° 46'
E48-32A	32° 48'	83° 52'
E48-31A	34° 49'	84° 08'
E48-18	35° 26'	91° 53'
E48-28A	38° 33'	79° 55'
E48-23	39° 31'	83° 43'
E48-03	41° 01'	100° 00'
E50-03	42° 01'	100° 00'
E49-46A	44° 04'	100° 01'
E45-79	45° 03'	114° 22'
E45-77A	46° 26'	114° 25'
E49-42A	47° 20'	100° 00'

Closed-form Fourier series analysis is a mathematical expression of cosines used to describe any shape or any two-dimensional shape outline to a specified accuracy¹⁸. Fourier series both provides exact characterization of shape and separates the components of the whole form, enabling us to see how each component (harmonic) affects the total shape. Each harmonic is an independent and uncorrelated measure of a specific geometric contribution to the total shape^{14,19}. The shape of each specimen is represented by a series of harmonics of the form: $R_n \cos(n\theta - \phi_n)$, where R_n represents the amplitude of the n th harmonic, and the remainder concerns the phase angle of that harmonic. The set of harmonic amplitudes constitutes the 'harmonic amplitude spectrum' (Fig. 1a). The Fourier series is estimated for each planktonic foraminiferal projection from analysis of the coordinates of peripheral points with the origin at the centre of gravity of the foraminifera (see ref. 18). Each harmonic amplitude represents the relative contribution to the empirical shape of a characteristic shape component. For example, the amplitude of the second harmonic represents the contribution to the empirical shape of a 'figure eight' and is thus a measure of elongation. The third harmonic represents a trefoil, the fourth a quadrefoil, and so on. In general, the n th harmonic amplitude represents the shape contribution of an n -leaved clover¹⁸. The observed shape is thus partitioned into a series where gross shape (for example, elongation, and triangularity) is measured by the harmonic amplitudes of the lower harmonic orders, and increasingly fine-scaled surface sculpture is measured at higher orders.

Fourier analysis in closed form, given enough terms, has enough information to describe any shape completely. This is demonstrated in Fig. 2 by computing the Fourier series of amplitudes and phase angles for a given shape, and then using only the information contained in the Fourier series to reconstruct the shape. For example, in Fig. 2, composite shapes are formed for a specimen of *G. truncatulinoides* by the successive addition of the first 20 terms in the computed Fourier series until the original shape is accurately reproduced.

Table 2 χ^2 Contingency table*

Harmonic no.	χ^2 Results
2	15.3†
3	49.9†
4	25.2†
5	4.43
6	30.06†
7	6.5
8	10.69
9	10.68
10	9.63

* Calculations were performed on two end member samples: E48-11A and E49-42A (Table 1).

† Values that exceed critical χ^2 value (0.1) of 13.3.

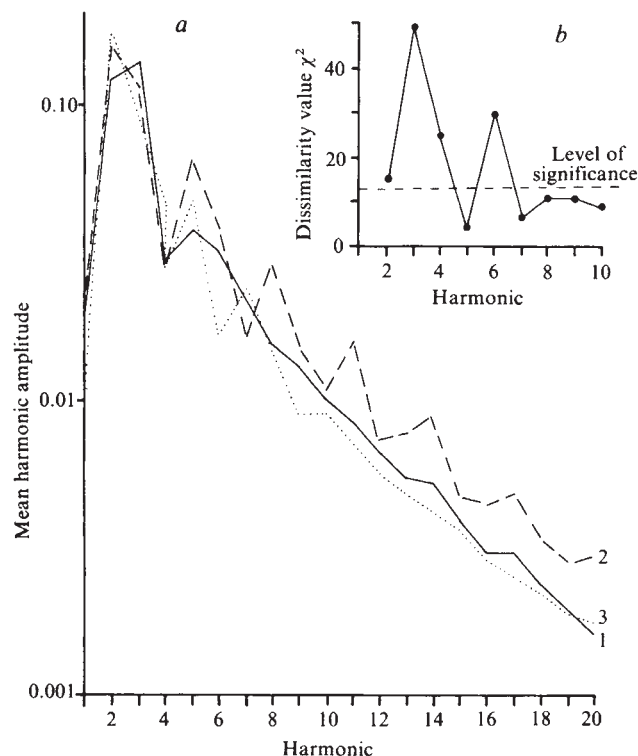


Fig. 1 a, Mean harmonic amplitude spectra of *G. truncatulinoides* from (1) V20-175, (2) E48-28A and (3) E49-42A (Table 1); b, plot of χ^2 values indicating the significance of harmonics 2, 3, 4 and 6.

The specimens of *G. truncatulinoides* used in this Fourier biometric study were removed from the surface sediment of 11 trigger cores and four piston cores from the southern Indian Ocean (lat 22–47° S) (Table 1). The principal oceanographic features of the surface waters in this region are the subtropical convergence (38–40° S) separating the subtropical and sub-Antarctic waters, and the polar front (48–50° S) separating sub-Antarctic and Antarctic waters. Both fronts are defined by strong salinity and temperature gradients²⁰. Only samples which contained well preserved Recent or late Holocene foraminifera were chosen^{21,22}. An aliquot was removed from each sample using an FB Dial microsplitter and ~35 foraminiferal specimens were selected from the 300–450 μ m size fraction. A separate experiment was performed to monitor the effects of size on test shape²³. The coiling direction of the assemblages varied from 100 to 6% sinistrally coiled. Fourier analysis was performed on the side view of all specimens. A normalization process in the Fourier series means that differences in size do not affect shape comparisons. The periphery of each foraminifera specimen was digitized as 48 equiangular coordinates and then converted to a Fourier series containing 20 harmonics. As seen in Fig. 2, the first few harmonics describe the gross shape while the higher-order harmonics refine the overall test shape. The result is a harmonic amplitude spectrum for each foraminiferal specimen with changes in shape reflected by changes in amplitude values. The amplitude spectrum for a sample comprises the primary data used for analysis (Fig. 1a). Each foraminifera in a sample contributes an amplitude value for every term of the series, that is the 20 terms calculated produce an $n \times 20$ matrix (n = number of foraminifera). Thus, for each term of the series, histograms (Fig. 3) can be constructed by plotting amplitude values (Fig. 1a) as a function of frequency of occurrence. The histograms are called shape frequency distributions. Each foraminifera can be described by 20 harmonic amplitude values, so each sample can be described by 20 shape frequency distributions (Fig. 3).

Thus, with the *G. truncatulinoides* data in this form, samples can be statistically compared and the specific harmonics

Table 3 Correlation coefficients from linear regression analyses

	Harmonic 2 (elonga- tion)	Harmonic 3 (conical shape)	Surface tempera- ture (°C)	Coiling % sinistral	Lat
Harmonic 2	1.00	-0.46	-0.70	0.87	0.76
Harmonic 3	-0.46	1.00	0.77	-0.42	-0.73
Temperature (°C)	-0.70	0.77	1.00	-0.76	-0.99
Coiling	0.87	-0.42	-0.76	1.00	0.81
Lat	0.76	-0.73	-0.99	0.81	1.00

Values are results from Fourier shape analysis of *G. truncatulinoides*.

responsible for shape differences can be determined. A χ^2 table was constructed for the first 10 harmonics as these harmonics capture the significant components of overall shape in this species (Fig. 1b, Table 2). According to Benzecri²⁴, χ^2 values can be used as indicators of dissimilarity. Plots of χ^2 values indicate that the 2nd, 3rd, 4th and 6th harmonics are statistically significant in describing the shape of this species (Fig. 1b). Because the harmonic amplitude spectra indicated that harmonics 2 and 3 contain the highest degrees of shape information (Fig. 1a), these are used in the following analysis.

Shape frequency diagrams for harmonics 2 (elongation) and harmonic 3 (triangularity or conical shape) show the amplitude distributions in all 15 samples ranked according to latitude (Fig. 3). The conical shape of *G. truncatulinoides* (harmonic 3) increases from the subpolar to subtropical water masses, in agreement with earlier biometric studies of this species^{16,17}. The frequency distributions of harmonic 2 (elongation), however, do not show a clear relationship with latitude. Instead, elongation seems related to the coiling direction of the populations (Fig. 3). In populations dominated by sinistrally coiled individuals (80–100% sinistral) south of 31° S, the mean amplitude values do not change systematically and show no variation in the elongation of *G. truncatulinoides*. In populations with significant numbers of dextrally coiled individuals (42–94% dextral) (30–22° S), *G. truncatulinoides* becomes less elongated. This relationship has never before been observed in this species.

Linear regression analyses were performed on harmonics 2 and 3 in relation to coiling ratio and various environmental parameters (Table 3). Harmonic 2 is strongly correlated with coiling direction ($r = +0.87$). Elongation is also correlated with latitude and surface-water temperature but not at the level of significance as with coiling-direction preference. Harmonic 3, on the other hand, has its highest correlation with surface-water temperature ($r = 0.77$) and a poor correlation with coiling ($r = 0.42$). We interpret the correlation between triangularity and water temperature to be indicative of ecophenotypic variation, that is, environmental-induced variation. The elongation (harmonic 2) of *G. truncatulinoides* may reflect more phenotypic

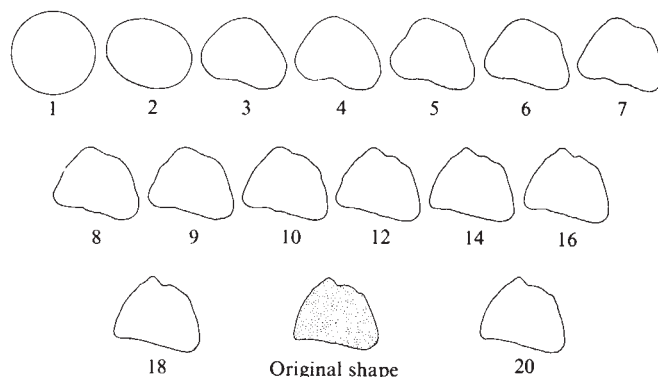


Fig. 2 Reproduction of the shape of *G. truncatulinoides* (from V20-175) by addition of successive harmonics. The original shape is reproduced using the first 20 harmonics.

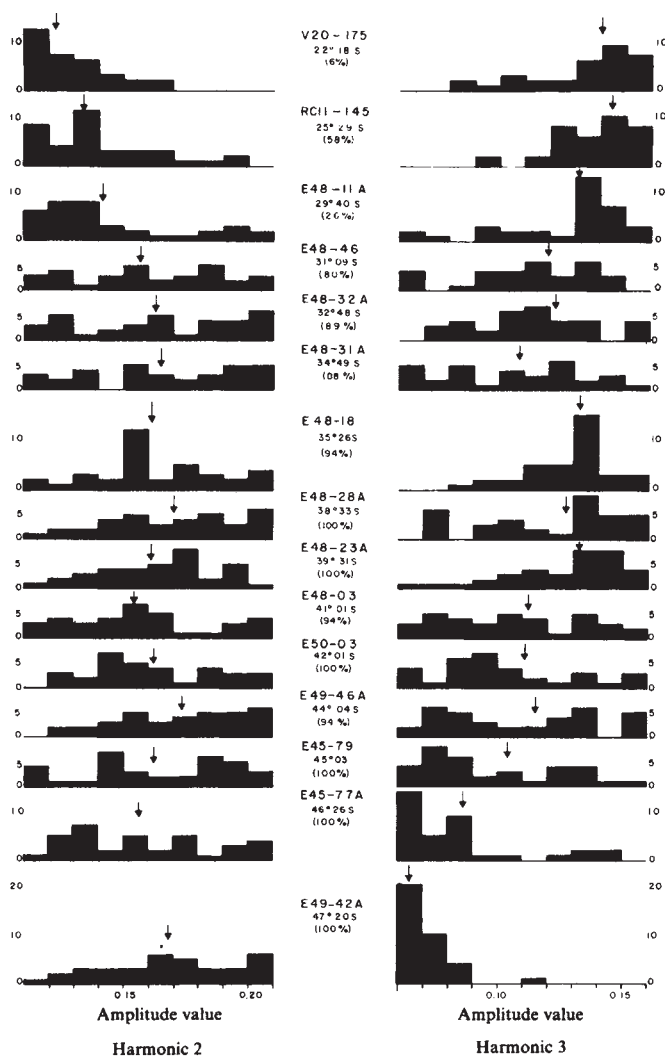


Fig. 3 Histogram plots of harmonic amplitude values for harmonic 2 (elongation) and harmonic 3 (triangularity/conical shape) in *G. truncatulinoides*. The histograms are ranked according to latitude and the per cent sinistrally coiled specimens in each sample is also shown. Arrows indicate the mean harmonic amplitude values in each sample.

variation (that is genetic and environmental influence) than ecophenotypic variation if we assume that coiling direction is genetically controlled. The factors governing coiling in foraminifera are not well understood². However, the coiling of *G. truncatulinoides* in the southern Indian Ocean may reflect more of a clinal relationship rather than a strict environmental relationship, because this species remains dominantly sinistrally coiled across such an important oceanographical and biogeographical boundary as the subtropical convergence.

This study clearly illustrates that the conical shape of *G. truncatulinoides* varies as a function of water temperature and, as such, can be used as a palaeoceanographical index of water temperature in this region of the southern Indian Ocean. Current work uses the conicalness/ water temperature correlation to estimate water mass changes in the southern Indian Ocean sediments during the late Quaternary. The significance of the relationship between test elongation and coiling direction can only be resolved by studies of living plankton and laboratory-cultured specimens. We have demonstrated, however, that Fourier shape analysis is an accurate and quantitative method for rapid determination of the components of test shape in foraminifera. Because test shape may vary as a function of phylogenetic (evolutionary) affinities, environmental stress and ecophenotypic variation, we believe that Fourier shape analysis

could be a powerful tool in morphometric studies of Cenozoic and late Mesozoic foraminifera.

We thank James P. Kennett for helpful criticism, Robert Ehrlich for helpful discussions, Cary Fico for his assistance with the Fourier software and Duane Eppler for use of his Drawshape program. This research was supported by NSF grant DPP77-21929 and a University of South Carolina Faculty and Productive Scholarship Grant. Belle W. Baruch Institute for Marine Biology and Coastal Research contribution no. 376.

Received 18 August; accepted 31 October 1980.

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Parental investment in male and female offspring in polygynous mammals

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Two evolutionary theories predict how mammalian parents would be expected to allocate the resources at their disposal to their male and female progeny. Where reproductive success varies more widely among males than females and variation in success among adults is influenced by parental investment¹, parents would maximize their reproductive success by allocating a greater proportion of their resources to individual sons than to individual daughters²⁻⁵. However, because the benefits of producing offspring of one sex are inversely related to the total investment allocated to them, parents should, on average, divide their total investment equally between their male and female progeny, rearing fewer of whichever sex is individually more expensive to produce⁶⁻¹⁰. Here we examine the extent to which parental investment in red deer (*Cervus elaphus*) and other polygynous mammals matches these predictions. We conclude that, in several mammals, mothers invest more heavily in individual sons than daughters but that, contrary to prediction, there is no indication that fewer male offspring are reared in these species.

In most polygynous mammals, lifetime reproductive success probably varies more widely among males than females and is affected by maternal investment¹. For example, in the red deer of the Isle of Rhum (Scotland), where hinds live for 12-14 yr, mating in October, giving birth in June and weaning their calves

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Table 1 Birth sex ratios of calves born between 1970 and 1979 to different categories of red deer hinds

Age of mother (yr)	3	4	5-6	7-8	9-10	11-12	13+
% Males	52.6	59.7	56.0	58.3	53.8	58.8	69.0
<i>n</i>	38	67	109	84	65	57	29
Reproductive status in previous year	Failed to produce calf		Calf died within month of birth		Calf reared through first winter		
% Males	59.3		60.3		56.4		
<i>n</i>	59		68		218		
Quality of home range	Superior				Inferior		
% Males	56.0				57.1		
<i>n</i>	168				98		

For details of data collection and allocation see ref. 13.

approximately 6 months later¹¹⁻¹³, individual mothers rear from 0 to 12 calves surviving to 1 yr old during their lifetime¹⁴. In contrast, stags, which hold large harems of hinds during the mating season, father from 0 to >25 calves surviving to 1 yr during their lifetime^{14,15}. In both sexes, reproductive success is probably affected by maternal investment during the growth period, but the relationship is particularly close among stags, where growth during the first year of life is related to adult body size and fighting ability¹⁴.

In precisely these circumstances, mothers should maximize their reproductive success by investing more heavily in individual sons than daughters. Do they do so? One way they might achieve this would be to vary the sex ratio of their progeny according to their own body condition, producing relatively more male offspring when they were in good condition (and could afford to invest heavily) and relatively fewer males when in poor condition, perhaps by selectively aborting or resorbing male fetuses². However, there is little evidence that factors which affect maternal condition in red deer have an important influence on birth sex ratios, which do not differ significantly between mothers of different ages, between those that have suffered the energetic strains of rearing a calf through the previous winter and those that have not done so, or between those occupying superior compared with inferior home ranges, although sample sizes are admittedly small (Table 1). Moreover, despite claims to the contrary², evidence for an association between maternal condition and birth sex ratio in other mammals is ambiguous^{16,17}: some studies have found a tendency for females in good condition to produce relatively more males¹⁸⁻²³, while others have found the reverse²⁴⁻²⁷.

An alternative possibility is that the sex ratio of progeny does not vary with maternal condition but that mothers transfer a greater proportion of their body reserves to sons than to daughters³⁻⁵. Several lines of evidence suggest that this is the case in red deer: compared with females, male calves have significantly longer gestation lengths (236.1 days versus 234.2 days, $P < 0.05$), heavier birth weights (6.72 kg versus 6.23 kg, $P < 0.002$) and, by analogy with other studies of dimorphic deer²⁸⁻³¹, probably gain weight faster. Observations show that male calves suck from their mothers more frequently (5.1 ($n = 17$) versus 2.6 ($n = 10$) daily suckling bouts: $P < 0.05$) and there is a trend for the duration of suckling bouts involving male calves to be longer than those involving females (Fig. 1). Evidence that the costs of rearing male calves exceed those of rearing females are provided by comparisons of the subsequent reproductive performance of mothers: between 1971 and 1980, hinds that had reared a male calf through the previous winter were less likely to produce a calf than those which had reared a female (Wilcoxon matched pairs signed ranks test across years, $T = 11$, P (one-tailed) = 0.05). In addition, hinds calved on average 11 days later following winters when they had supported a male calf compared with those when they had supported a female (Wilcoxon matched pairs signed ranks test across individual hinds, $z = 2.66$, $n = 32$, $P < 0.01$).

As calving date is known to be related to body condition in red deer^{32,33} and late calving increases the chances that calves will die during their first winter³⁴, this provides additional evidence of the increased costs of rearing male calves.

Similar data for other species are uncommon. However, detailed studies of maternal investment in elephant seals (*Mirounga angustirostris*) have found that male pups are weaned later than females³ and, age for age, tend to be larger, while in a variety of other polygynous mammals, males commonly show longer gestation lengths, heavier birth weights, faster growth rates and greater food requirements than females^{4,35-42}. There is also some evidence that the energetic costs of producing and rearing males may exceed those of producing and rearing females: in sheep, lambs born co-twin with males are lighter than those born co-twin with females⁴³ and, in Indian cattle, two studies have found a (non-significant) tendency for the next conception to be delayed longer after a bull calf has been produced^{44,45}. However, a contrasting situation is found in colonies of macaques, where dominant females who have produced daughters are significantly less likely to produce another offspring in the next season than those that have reared sons⁴⁶. One possible explanation is that, in this case, parental investment may have a greater effect on the breeding success of daughters and less on that of the sons because daughters generally inherit their mother's dominance rank while male body size may have little effect on competitive success⁴⁷.

In species where sons cost more to produce and rear than daughters, the sex ratio of progeny should be biased against males by the age at which parental investment is terminated⁸. However, sex ratios at weaning (as well as at birth) are often male-biased in polygynous species⁴⁷. In particular, slightly male-biased weaning ratios occur in both species for which there is good evidence of increased investment in males before weaning (Table 2).

There are at least two ways in which these results could be reconciled with current theory: (1) Parental investment in daughters (but not sons) continues after weaning, balancing heavier pre-weaning investment in sons. This may be the case in a number of species where daughters adopt territories or home ranges overlapping those of their mothers while sons disperse^{4,49}: if the presence of resident daughters reduces the amount of food available to the mother and her subsequent offspring, maternal tolerance of mature offspring should be regarded as a continuation of parental investment. However, this argument is unlikely to explain the existence of male-biased weaning ratios in either red deer or elephant seals. In the latter, both sexes of offspring are abandoned by their mothers on the breeding beaches and there is no evidence of social bonds between mothers and their weaned offspring^{3,48}. In the former, toleration of mature daughters is unlikely to represent an important form of investment since unrelated hinds occupy overlapping home ranges, so that if a mother were to exclude her daughters from her range, this would have little effect on the resources available to her. (2) Alternatively, the primary sex

Table 2 Sex ratios at birth and after weaning in two polygynous mammals

	Sex ratio at:	
	Birth	12 months (~6 months post-weaning)
Red deer ¹⁴ (<i>Cervus elaphus</i>)		
% males	57.0	53.3
n	423	296
	Birth	1 month (shortly after weaning)
Northern elephant seal ⁴⁸ (<i>Mirounga angustirostris</i>)		
% males	51.6	51.3
n	2,396	2,052

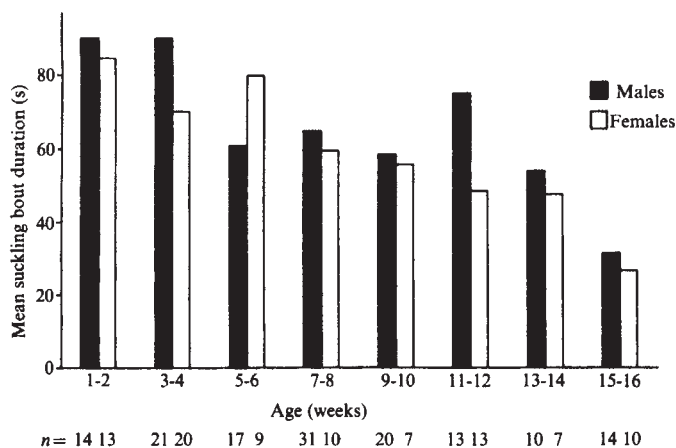


Fig. 1 Duration of suckling bout(s) to male and female calves between July and October.

ratio may be fixed at unity. In this case, selection for increased investment in individual sons could prefer parents that allocated a greater proportion of their total investment to male progeny⁵. However, evidence that sex ratios can be modified argues against this theory⁴⁷.

Thus, the available data suggest that polygynous mammals commonly invest more in individual sons than daughters and that this tendency may be associated with a greater total investment in male than female progeny. Why total investment is not equalized between males and females remains an anomaly.

We thank Rosemary Cockerill, Professor J. Maynard Smith, Professor R. V. Short and Dr Paul Harvey for advice and criticism and the NERC, SRC and Leverhulme Trust for support.

Received 23 May; accepted 20 November 1980.

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Photon-like signals following weak rhodopsin bleaches

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The rod visual system in its dark-adapted state behaves as a near-ideal light detector¹. Psychophysical studies on the reliability of light detection in man^{2,3}, analysis of the dark noise in rod bipolar cells⁴ and observation of photon-like events in rods⁵ in the dark suggest that the visual pigment, rhodopsin, is very stable against spontaneous isomerization. When a light which bleaches a small fraction of the rhodopsin is extinguished, the visual threshold may be increased by several orders of magnitude. The eye is then 'light-adapted' and the process (or processes) by which the sensitivity returns constitutes dark adaptation. We report here that bleaching of a small fraction of the rhodopsin produces a prolonged increase in the noise observed in the dark in rod bipolar cells of the dogfish retina. The associated noise events are similar to those produced by the absorption of light quanta and presumably have their origin in the rods which transmit their signals to the bipolar cells. This increased noise after bleaching would decrease the reliability of detection of light quanta and contribute to the elevation of visual threshold.

Responses to light were recorded intracellularly in rod bipolar cells in the initially dark-adapted, hemisected eyecup of the dogfish, *Scyliorhinus canicula*. The bipolar cells studied here were those which in the dark-adapted state depolarized in response to light delivered as full-field illumination. The methods of recording, identification of cells and estimation of irradiance and bleaching have been described previously^{6,7}. Because of the high voltage gain in signal transmission from rods to bipolar cells⁷, we could study responses to light and changes in voltage noise during and following light which bleached fractions of between 10⁻⁹ and 10⁻³ of the rhodopsin.

Figure 1a shows the noise increase during dim light, bleaching, on average, about one rhodopsin molecule per rod in 50 s. The noise at higher gain and faster sweep during the period indicated by arrows is shown as the middle record of Fig. 2a. Power-spectral analysis of this noise suggests that most of the noise arises from random absorption of light quanta by the rods within the receptive field of the bipolar cell ('photon noise')⁴. When the dim light was switched off, there was no detectable change in the dark noise.

Figure 1b shows the response of the same cell to light 4,000 times brighter, during which time the dark noise was suppressed

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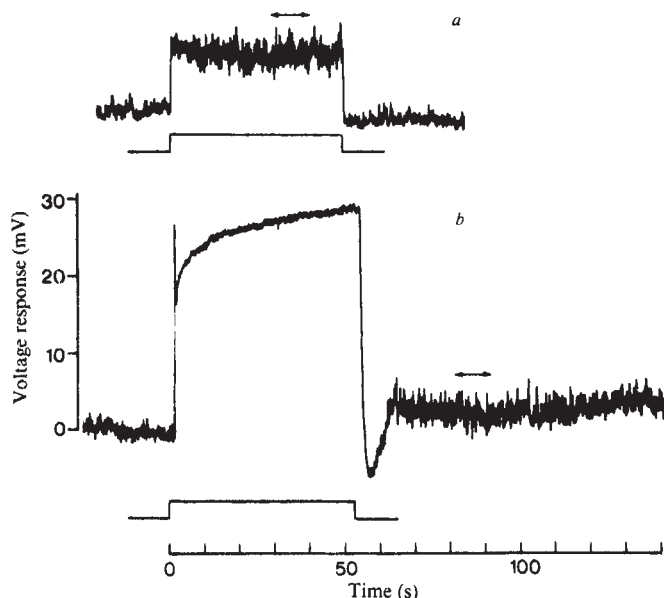


Fig. 1 Voltage responses of a rod bipolar cell to steps of light, accompanied by an increase of noise during very dim illumination (*a*) and a prolonged increase of noise following a bleach (*b*); both records are from the same cell. The duration of the light stimulus is indicated under each record, which in all experiments was delivered as full-field illumination. *a*, The total bleach during the step was 1.1 Rh^{**} , at a rate of $0.022 \text{ Rh}^{**} \text{ s}^{-1}$, where Rh^{**} signifies a rhodopsin molecule bleached per rod⁷. The voltage noise variance in the dark (corrected for noise due to the electrode determined with the electrode in the cell in a bright light) was 0.20 mV^2 ; the noise variance during illumination was 0.95 mV^2 . *b*, The response to a step of light with a total bleach of $4,300 \text{ Rh}^{**}$ at a rate of $79 \text{ Rh}^{**} \text{ s}^{-1}$. Assuming 3×10^8 rhodopsin molecules per rod (ref. 4), this corresponds to a 0.0014% bleach. Note that the noise increased with a delay following the light step. The noise variance following the bleach (during the period indicated by arrows) was 1.0 mV^2 . Potential of the cell in the dark, -56 mV ; maximum response to a bright flash, 30 mV ; flash sensitivity $1,860 \text{ mV}$ per Rh^{**} (ref. 7); step sensitivity, 645 mV per Rh^{**} (ref. 7). Temperature, 17.5°C . An increase in noise was observed in 7 out of 10 cells following light steps producing bleaches of 10^{-6} – 10^{-5} of the rhodopsin. Low sensitivity and noise in the recording system may have obscured the effect in some cells.

and then increased with a delay of about 10 s when the light was switched off. Several features of Fig. 1*b* require comment. The non-steady depolarization during light may be due to a bipolar cell membrane non-linearity, while the slow, upward creep of the depolarization during steady light may indicate a slow inactivation of potassium conductance. Although this membrane non-linearity may contribute to a decrease in noise during light which produced response saturation, the decrease in noise can be shown to be due mainly to the non-linear relation between light intensity and depolarization. Only in the linear region of response does photon noise variance increase in proportion to the mean depolarization. As saturation of the response is approached, the noise variance declines to zero. In Fig. 1*b*, the light bleached 4,300 rhodopsin molecules per rod or about 0.0014% . When it was turned off, there was a transient hyperpolarization followed by a prolonged after-depolarization, accompanied by an increase in noise variance. The after-depolarization reached its peak value of 5 mV and the noise variance increased to six times its dark value 10 s after the light was turned off. The noise variance returned to its initial dark value in about 3 min. The post-bleach noise during the interval indicated by arrows in Fig. 1*b* is shown at an expanded sweep and amplification in Fig. 2*a* (bottom record). Clearly, the character of the post-bleach noise is similar to that during dim light and this similarity to photon noise was confirmed by their power spectra.

The effect of light bleaching 0.17% rhodopsin is illustrated in Fig. 3, recorded in another cell. As in the cell of Fig. 1, the noise increased after a delay and the increased noise was associated with a depolarization lasting about 10 min. The noise recorded about 4.5 min after the light was switched off is shown at an expanded sweep and higher gain as the bottom record of Fig. 2*b*. The post-bleach noise had characteristics similar to the noise recorded during dim light in the same cell (the middle record of Fig. 2*b*). The power spectra of the noise recorded at different times during the period following the bleaching were all indistinguishable (except for a power-scale factor) and were the same as during dim light. Because the events giving rise to the increased noise are like photon events, we presume that they arise in the rods. Recently, Lamb has observed increased fluctuations in single rods, resembling light quantal events, which are induced by bleaching⁸.

We can estimate the equivalent rate of photon-like events in the rod in the following way. For a linear shot process model for

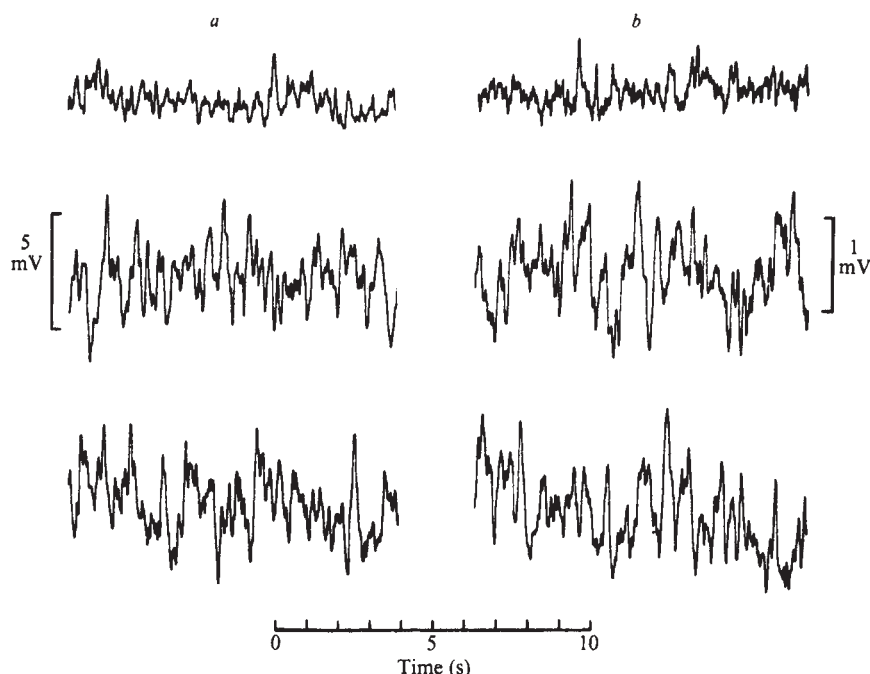


Fig. 2 Voltage noise in bipolar cells in darkness (upper row), during dim illumination (middle row) and following bleaches (bottom row), at higher gain and sweep speeds. *a*, Records from the cell illustrated in Fig. 1. The middle and lower records are from the intervals indicated by the arrows in Fig. 1*a* and Fig. 1*b*, respectively. *b*, Records from the cell illustrated in Fig. 3. The middle record shows the noise during dim illumination at an intensity of $0.026 \text{ Rh}^{**} \text{ s}^{-1}$ (~ 1 molecules bleached per 40 rods per s), which depolarized the cell by 2.7 mV. The bottom record shows the noise following a 0.17% bleach, corresponding to the interval arrowed in Fig. 3.

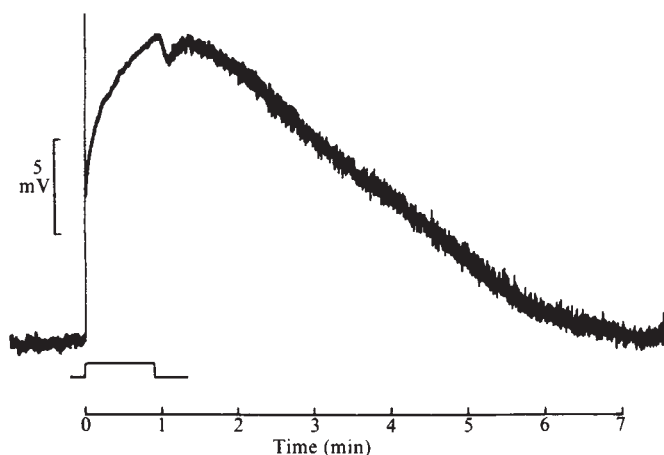


Fig. 3 Prolonged increase of noise in a rod bipolar cell following a pigment bleach of 0.17%. The rate of bleaching was $9.4 \times 10^5 \text{ Rh}^{**} \text{ s}^{-1}$ so that the total bleach was $5.2 \times 10^5 \text{ Rh}^{**}$. The noise variance in the dark (corrected for electrode noise) was 0.01 mV^2 . Approximately 4.5 min after the bleaching light was turned off, the noise variance was 0.074 mV^2 . The cell was held for 15 min following the bleach during which time the potential and noise returned to their pre-bleach values. Potential of the cell in the dark, -29 mV ; maximum response to light, 17 mV ; flash sensitivity, $320 \text{ mV per Rh}^{**}$; step sensitivity, $120 \text{ mV s per Rh}^{**}$. Temperature, 17°C .

events seen in the bipolar cell, the equivalent rate per rod would be

$$\lambda = V/(aN\tau)$$

where V is the mean depolarization, a is the amplitude of the shot event in the bipolar cell, N is the number of rods in its pool and τ is the integration time. (If, like the effect of light, the relation between mean depolarization and the rate of events is a rectangular hyperbola, λ would need to be corrected by a factor, $\{1 - (V/V_{\text{max}})\}^{-1}$, where V_{max} is the maximum response.) The product, $aN\tau$, is the step sensitivity and was determined in the well dark-adapted state as the slope of the relation between steady-state depolarization and light intensity in the linear range of response⁷. For the cell of Fig. 1, the peak rate of events, following a bleach of 0.0014% giving a 5-mV depolarization, was 0.01 per s per rod. This was 10.3 times the initial dark-adapted rate of equivalent events, determined in the way described by Ashmore and Falk⁴. Another bleaching exposure in the same experiment, bleaching 1/15th of that illustrated in Fig. 1b, resulted in an increased post-bleach noise variance of 70% greater than that of the initial well dark-adapted state. For the bleach of 0.17% illustrated in Fig. 3, the peak post-bleach rate (corrected for non-linear summation) amounted to 1.8 events per s per rod, which was 1,500 times the initial dark-adapted rate. These results suggest that the peak extra rate of noise events induced by bleaching is proportional to the amount of rhodopsin bleached, at least for small bleaches. Quantitatively,

$$\frac{\lambda_B}{\lambda_D} = \alpha R$$

where λ_B is the peak rate of additional events induced by bleaching, λ_D is the dark-adapted rate of equivalent events, R is the number of rhodopsin molecules bleached per rod and α the constant of proportionality amounting to $2-3 \times 10^{-3}$ per Rh^{**} , where Rh^{**} denotes one rhodopsin molecule bleached per rod.

These relatively weak bleaches give rise to an increased rate of spontaneous events lasting many minutes and the question arises of how the total number of extra events is related to the number of rhodopsin molecules bleached. Integration of the increased rate over time indicates that bleaching of a rhodopsin molecule has only a small probability, less than 0.001, of inducing an event. It is unlikely that this low probability is the result of regeneration of rhodopsin which in the elasmobranch eye⁹ is very much slower than the rate of recovery of the noise to its dark-adapted value.

The present results confirm Rushton's¹⁰ and Barlow's¹¹ conjecture that bleaching of rhodopsin in rods induces signals which constitute a background of 'dark light' or noise which would raise the threshold for detection of a light stimulus. Rushton¹⁰ found that the equivalent background light increased exponentially with the fraction of rhodopsin in the bleached state. Extrapolated to weak bleaches, the exponential relationship becomes one of linearity. However, note that the value of α reported here for weak bleaches is several orders of magnitude greater than that inferred from the psychophysical measurements^{10,11} with strong bleaches. Likewise, the rise in visual threshold in man following weak bleaches^{12,13} is also greater than that predicted from near-total bleaching.

An increase in the rate of occurrence of discrete events following bleaching may be general among photoreceptors for it has been reported to occur in the photoreceptors of invertebrates^{14,15}. We cannot decide yet whether the increased rate of spontaneous events in rods following absorption of light quanta is due to an increased rate of thermal isomerization of rhodopsin or to a link with some reaction involved in phototransduction.

The work was supported by an MRC project grant.

Received 29 August; accepted 19 November 1980.

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Isolation of the phagocytosis-inducing IgG-binding antigen on senescent somatic cells

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To remove senescent red blood cells (RBCs) from the circulation, macrophages must distinguish them from mature RBCs. That is achieved by a specific recognition system^{1,2}. An antigen that develops on the surface of a senescent RBC is recognized and bound by the Fab region¹ of an IgG autoantibody in the serum². Subsequently the Fc region of the autoantibody is recognized and bound by a macrophage³, which proceeds to phagocytose the RBC. The antigenic molecule can be extracted from senescent but not young RBCs with Triton X-100 (ref. 4), although 10-30% as much antigen can be extracted from middle-aged as from senescent RBCs⁴. I have now used IgG autoantibodies eluted from senescent RBCs to isolate and purify the IgG-binding antigen on senescent RBCs, and to detect the antigen on other somatic cells. The antigen is a $\approx 62,000$ -M_r protein which is present on stored platelets, lymphocytes and neutrophils, and on cultured human adult liver and embryonic kidney cells, as well as senescent RBCs.

IgG was eluted from human senescent RBCs⁵, by the method of Kochwa and Rosenfield⁶ (for details see Fig. 1). The IgG-containing eluates of senescent RBCs from 50 l of blood were pooled, and IgG isolated from the eluates by affinity chromatography with Protein A-Sepharose 4B (Pharmacia)¹.

Glycine-HCl eluates from the affinity column contained IgG and an additional peptide that was not part of the IgG molecule, as determined by polyacrylamide gel electrophoresis on gradient gels (Fig. 1). This peptide has a M_r of $\approx 62,000$ and migrates between IgG heavy chain and its dimer on polyacrylamide gels and is not present in IgG isolated from serum with Protein A columns (Fig. 1). This peptide stains with dansyl

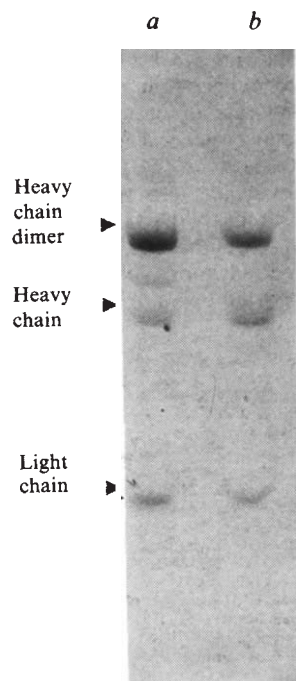


Fig. 1 Polyacrylamide gel electrophoresis of IgG eluted from senescent RBCs and purified with Protein A affinity columns. *a*, IgG eluted from senescent RBCs and purified with a Protein A-Sepharose column; *b*, IgG isolated from normal human serum by ion-exchange chromatography followed by Protein A-Sepharose chromatography. Blood was obtained from 250 healthy individuals between the ages of 21 and 35 yr, and was processed the same day that it was obtained. The blood was centrifuged for 15 min at 2,000g and the serum and white cells removed. RBCs were separated into populations of different ages by centrifugation^{4,5,8}, and young RBCs were removed. The remaining RBCs were washed three times with 50–100 vol of Dulbecco's phosphate-buffered saline (PBS), pH 7.4. IgG was eluted as described previously^{1,6}. Briefly, RBC membranes (ghosts) were prepared by digitonin lysis, washed three times with PBS, and IgG eluted with 0.1 M glycine-HCl buffer, pH 2.3. Eluates were neutralized with 1 M NaOH and concentrated to 0.25–1.00 ml using an Amicon Diaflow with a PM 10 filter. Solid material was removed by centrifugation at 27,000g. Samples were stored at -80°C until use. They were then thawed, pooled and incubated with Protein A-Sepharose 4B for 30–60 min at 37°C and 24–48 h at 4°C . The Protein A-Sepharose was poured into a 12-ml column prepared from a syringe and washed with 100 vol of PBS, then 100 vol of $10\times$ PBS followed by 100 vol of PBS. IgG was eluted with 2 vol of glycine-HCl buffer, pH 2.3, neutralized with 1 M NaOH and dialysed against distilled water (*a*). The amount of IgG obtained was 7.42 mg. Polyacrylamide gel electrophoresis was performed with 2.7-mm thick gradient slab gels containing 4–30% acrylamide. The buffer system of Laemmli was used⁹. Gels were stained for glycoproteins with dansyl hydrazine following the method of Eckhardt *et al.*¹⁰ with the modifications of Lutz *et al.*¹¹. Proteins were stained with Coomassie blue (shown here). Serum IgG was isolated by ion-exchange chromatography on DEAE-cellulose (DE 52, Whatman)^{1,2} followed by incubation with Protein A-Sepharose as described above (*b*).

hydrazine and thus is probably a sialoglycopeptide (not shown). Elution of the $\approx 62,000$ - M_r peptide from Protein A columns along with the IgG obtained from senescent cells suggests that it may be the senescent cell antigen for IgG autoantibodies.

As the peptide that co-purifies with the IgG eluted from senescent cells seems to be a sialoglycopeptide^{1,2,4}, I attempted to isolate the senescent cell antigen for IgG autoantibodies from preparations of RBC sialoglycoproteins. IgG eluted from senescent cells and purified by affinity chromatography with

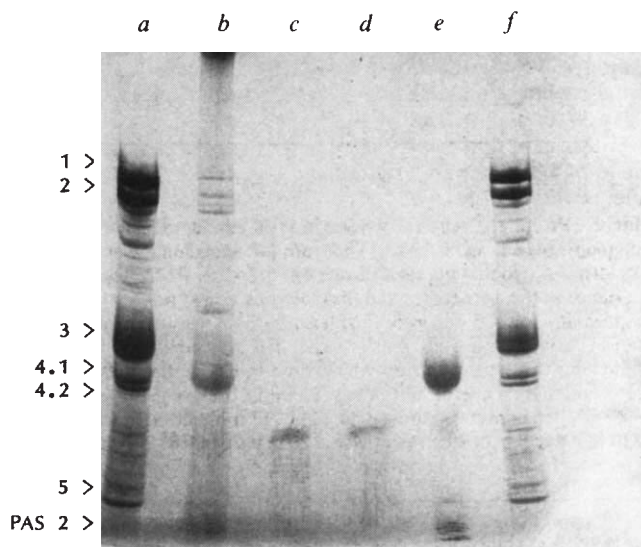


Fig. 2 Isolation of the antigen for IgG autoantibodies from human RBC sialoglycoproteins with affinity columns prepared from the IgG eluted from senescent RBCs. *a*, RBC ghost; *b*, Triton X-100 extract of ghosts; *c*, 62,000- M_r peptide isolated with senescent cell IgG affinity column from Triton X-100 extract; *d*, 62,000- M_r peptide isolated with the senescent cell IgG affinity column from lithium diiodosalicylate (LIS) extract of ghosts; *e*, LIS extract of ghosts; *f*, RBC ghost. Blood was obtained from healthy donors 21–30 yr old. Donor cells were typed for 27 known RBC blood group antigens, including ABO, Rh, MN, Ss, Duffy, Kell and Kidd. Donors did not have detectable autoantibodies to RBCs or antibodies to any known blood group antigen, as determined by the Immunohematology Laboratory, American Red Cross, nor did they have antibodies to nuclei, DNA, mitochondria, parietal cells or smooth muscle¹². All blood was processed the same day that it was obtained. RBCs from 500 ml of human blood were depleted of white cells and young RBCs by centrifugation on self-forming Percoll gradients^{4,8}. RBCs were washed three times in 50–100 vol of PBS with $75\ \mu\text{g ml}^{-1}$ phenylmethylsulphonylfluoride (PMSF) at 4°C . Right-side out RBC membranes were prepared by hypotonic lysis in 5 mM sodium phosphate buffer, pH 7.5, containing $50\ \mu\text{g ml}^{-1}$ PMSF¹¹. After five washes in 100 vol of the same buffer (*a* and *f*), ghosts were resuspended in a solution of 0.3 M LIS⁷ or 1.0% Triton X-100 and partitioned in a phenol-water mixture according to the procedure of Marchesi and Andrews⁷. The water phase was dialysed extensively against distilled water at 4°C and then lyophilized (*b*). The lyophilized material extracted with LIS was resuspended in 5 mM phosphate buffer and extracted with chloroform-methanol (2:1)¹³. The aqueous phase was dialysed against distilled water (*e*) and processed with the senescent cell IgG affinity column. Triton X-100 extracts were processed with the affinity column without chloroform-methanol treatment. The affinity column was washed with 100 vol of the following: PBS with $50\ \mu\text{g ml}^{-1}$ PMSF, $10\times$ PBS, 5 mM phosphate buffer, and then PBS with $50\ \mu\text{g ml}^{-1}$ PMSF. Material bound by the column was eluted with 15 vol of glycine-HCl, pH 2.3, neutralized, dialysed against distilled water at 4°C and lyophilized (*c* and *d*). Electrophoresis was performed using gradient gels containing from 2 to 16% acrylamide and the buffer system of Fairbanks *et al.*¹⁴, except that 0.2% SDS was used. Sample buffer: 10 mM Tris-HCl, pH 8.0, containing 1 mM Na EDTA, 40 mM dithiothreitol, 5% glycerol, 0.2% SDS and bromophenol blue. All of the proteins extracted with LIS stained with dansyl hydrazine. The photograph shows the gel stained with Coomassie blue. PAS, periodic acid-Schiff: designation for RBC sialoglycoproteins.

Protein A was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia). Columns were prepared and washed with 15 vol of glycine-HCl buffer, pH 2.3, to break the bond between the insolubilized IgG and its senescent cell antigen. After neutralization with NaOH, preparations of pure RBC membrane sialoglycoproteins, obtained by extraction with lithium diiodosalicylate and partition in a phenol-water mixture according to the procedure of Marchesi and Andrews⁷ (see Fig. 2 for details), were processed with the senescent cell IgG column. After extensive washing, the material on the column was eluted with glycine-HCl buffer, pH 2.3, neutralized, dialysed against deionized, distilled water at 4 °C, lyophilized and analysed by gel electrophoresis. Both dansyl hydrazine and Coomassie blue stains of the gels revealed a single band migrating at a M_r of $\approx 62,000$ which appears below band 4.2 in Fig. 2. When this experiment was repeated using 1% Triton X-100 extracts of RBC membranes⁸, the same results were obtained. A single peptide of $M_r \approx 62,000$ was observed on gels of the material eluted from the senescent cell IgG affinity column (Fig. 2). These experiments indicate that the $\approx 62,000$ - M_r peptide carries the antigenic determinants recognized by IgG obtained from freshly isolated senescent cells.

To confirm this finding, an erythrophagocytosis inhibition assay was performed^{1,2}. IgG was absorbed by incubation at 37 °C for 30 min with either the 62,000- M_r peptide eluted from senescent cell IgG affinity columns or with the sialoglycoprotein mixture that had not bound to these affinity columns. The

Table 1 Inhibition of the phagocytosis-inducing ability of IgG autoantibody eluted from senescent RBC by previous absorption with *a*, stored platelets, lymphocytes or neutrophils, and *b*, human adult liver cells or human embryonic kidney cells

	Cell type used for absorption	% Phagocytosis (mean $\pm$ s.d.)
<i>a</i>	None	37 $\pm$ 4
	Platelets	2 $\pm$ 2
	Lymphocytes	0
	Neutrophils	0
<i>b</i>	None	38 $\pm$ 6
	Liver	0
	Kidney	7 $\pm$ 7

In *a*, macrophages, lymphocytes, neutrophils and platelets were isolated by centrifugation on Percoll gradients⁴. Macrophages were cultured, and lymphocytes and platelets were stored in Alpha minimal essential medium with 10% fetal calf serum (FCS) at 4 °C (refs 2, 4). Citrate phosphate-dextrose solution was added to the platelet suspension to prevent activation. The specific IgG autoantibody eluted from senescent cells (6 μ g) was added to 1-ml suspensions of $\approx 2 \times 10^7$ lymphocytes or 1×10^8 platelets and incubated for 30 min at 37 °C and 30 min at 24 °C (refs 1, 2, 4). Positive controls consisted of autoantibody incubated with medium only. Cells were removed by centrifugation and the supernatant containing the remaining antibody was added to 5×10^8 stored RBCs. RBCs were incubated for 15 min at 37 °C and 1.5 h at 24 °C. Aliquots of each sample (0.1 ml) containing 5×10^7 RBCs were added to macrophage cultures. Phagocytosis was determined as described previously^{1,2,4,8}. Data are presented as the mean $\pm$ 1 s.d. The amount of IgG antibody used for absorption and the number of cells used were based on preliminary dose-response experiments. The phagocytosis-inducing ability of the senescent cell IgG could not be absorbed with freshly isolated young RBCs (43 $\pm$ 9% phagocytosis) but was abolished by absorption with stored RBCs (0% phagocytosis). Data are presented as the mean $\pm$ s.d. In *b*, human adult liver and human embryonic kidney (Chang and Flow 4000 respectively, Flow) cells were stored in liquid nitrogen, thawed and grown in tissue culture for 7 days before use. The media were changed 2 days after initiation of culture. The less viable non-adherent cells were collected by centrifugation and resuspended in 1 ml of Alpha minimum essential medium containing 10% FCS⁴. IgG eluted from senescent cells (3 μ g) was incubated with 2.1×10^6 liver cells (57% did not exclude Trypan blue) or 5×10^6 kidney cells (82% did not exclude Trypan blue) for 20 min at 37 °C and 2 h at 24 °C. Cells were removed by centrifugation, and the supernatant incubated with stored RBCs. The phagocytosis inhibition assay was performed as described in the text.

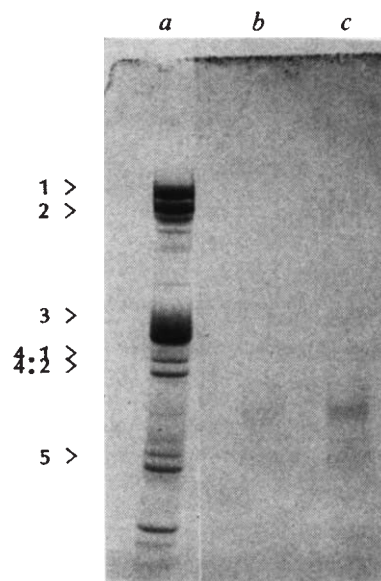


Fig. 3 Isolation of the antigen for IgG autoantibodies from human lymphocyte sialoglycoproteins with affinity columns prepared from the IgG eluted from senescent RBCs. *a*, RBC ghost; *b*, 62,000- M_r peptide isolated with the senescent cell IgG affinity column from LIS extract of RBC ghost; *c*, 62,000- M_r peptide isolated with the senescent RBC IgG column from LIS extract of lymphocyte membranes. Lymphocytes (8×10^8) were isolated from 500 ml of blood by centrifugation on Percoll gradients⁴. After three washes, membranes were prepared by hypotonic lysis in 5 mM NaPO₄ and 1 mM EDTA, pH 7.6 (ref. 15). Membrane suspensions were centrifuged at 248g to remove large particles. The supernatant containing the membranes was centrifuged at 27,000g. Membranes were washed three times in 100 vol of the same buffer, extracted with LIS and partitioned in a phenol-water mixture as described for RBCs in Fig. 2 legend. The aqueous phase was dialysed against distilled water and processed with the senescent RBC IgG affinity column.

absorbed IgG was then incubated with stored RBCs⁴ for 30 min at 37 °C because stored RBCs have the exposed receptor^{1,2,4}. The RBCs were washed three times and incubated with autologous macrophage cultures^{1,2,4}. RBCs incubated with the IgG absorbed with excess 62,000- M_r peptide were not phagocytosed (0% phagocytosis), indicating that no free antigen-binding IgG remained in solution. In contrast, 52 $\pm$ 3% ($\pm$ s.d.) of the RBCs incubated with IgG absorbed with the eluate that was not bound by senescent cell affinity columns were phagocytosed, indicating the presence of free antigen-binding IgG. The same amount of phagocytosis was observed when stored RBCs were incubated with IgG that was not absorbed (55 $\pm$ 6%). Thus, the 62,000- M_r peptide, but not the remaining sialoglycoprotein mixture from which it was isolated, abolishes the phagocytosis-inducing ability of IgG. This indicates that the 62,000- M_r peptide is the antigen which emerges on the surface membrane of cells as they senesce.

Other somatic cells were then examined for the presence of the antigen which appears on senescent RBCs. Macrophages, lymphocytes, platelets and neutrophils were isolated by centrifugation on Percoll density gradients^{4,9}. Cells were stored at 4 °C for 48 h. They were then washed and incubated with the autoantibody eluted from senescent cells (Table 1). Cells were removed by centrifugation and the supernatants containing any remaining IgG were incubated with stored RBCs. Stored RBCs were washed and incubated with macrophages. The phagocytosis-inducing ability of the autoantibody eluted from senescent cells was absorbed by stored lymphocytes, neutrophils and platelets (Table 1a).

Human adult liver cells and human embryonic kidney cells, grown in culture, were also examined for presence of the

antigen. The less viable cells which were no longer adherent were collected from culture supernatants by centrifugation (Table 1b). Absorption of the IgG eluted from senescent RBCs with both liver and embryonic kidney cells abolished its phagocytosis-inducing ability (Table 1b).

As the results of the phagocytosis inhibition assay experiments indicated that the antigen that appears on senescent RBCs also emerges on other somatic cells, I attempted to isolate the antigen from lymphocytes. Sialoglycoprotein mixtures were prepared from lymphocyte membranes in the same manner as described for RBCs (for details, see Fig. 3). Lymphocyte sialoglycoprotein mixtures were processed with the senescent RBC IgG affinity column. Gel electrophoresis of material eluted from the column with glycine-HCl buffer revealed a band migrating at a M_r of $\approx 62,000$, at the same position as the antigen isolated from senescent RBC (Fig. 3). This finding confirms the results obtained with the phagocytosis inhibition assay, indicating that

the antigen which appears on senescent RBC also appears on other somatic cells.

Emergence of the $\approx 62,000$ - M_r protein initiates binding of IgG autoantibodies *in situ* and phagocytosis of senescent cells by mononuclear phagocytes. This antigen is present on stored lymphocytes, platelets, and neutrophils, and on cultured liver and kidney cells. Therefore, the immunological mechanism² for removing senescent and damaged red cells may be a general physiological process for removing somatic cells, at least in mammals.

I thank Drs John Cook, Takashi Makinodan and Andrew Saxon for reviewing the manuscript, and Dr Moses Schanfield for advice and for providing blood-typing reagents, typing donor RBCs and screening donors for anti-RBC antibodies. This work was supported by a NIH grant. Workspace was provided by the Research Service, Wadsworth Medical Center.

Received 7 July; accepted 24 November 1980.

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Self-MHC-restricted cytotoxic T-cell response without thymic influence

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The thymus has a central role in directing the maturation and differentiation of T cells¹. These processes involve movement of cells of haematopoietic origin through the thymus and are supposed to require intimate physical contact between T lymphocytes and cells of the thymic epithelium. Cytotoxic T cells (CTLs) induced by virus-infected or hapten-modified autologous cells exhibit dual specificity: for the products of the self major histocompatibility complex (MHC, H-2 in the mouse) and for the foreign antigen²⁻⁴. It has been proposed that 'self' specificity of MHC-restricted CTLs is determined by the MHC-encoded antigens expressed on the surface of thymic epithelial cells⁵. We show here that the differentiation of MHC-restricted CTLs can take place without thymic influence. In these experiments, self-MHC-restricted CTLs differentiate from spleen cells of *nu/nu* mice which lack thymuses after stimulation with 2,4,6-trinitrophenyl (TNP)-coupled autologous cells in the presence of T-cell growth factor.

Spleen cells of C57BL/6 *nu/+* (*nu/+*) (H-2^b) and C57BL/6 *nu/nu* (*nu/nu*) mice were stimulated with TNP-coupled autologous cells *in vitro*. Cells were collected 5 days after stimulation and tested for lytic activity and specificity. An H-2-restricted cytotoxic T-cell response was generated from the cells of *nu/+* mice, whereas *nu/nu* mice did not respond to the antigen (Table 1). However, when T-cell growth factor (TCGF) (Table 1) was added to the cultures, *nu/nu* cells responded to

TNP-derivatized autologous cells. The cytotoxic cells killed preferentially TNP-derivatized autologous cells; TNP-coupled allogeneic cells (B10.BR, H-2^k) were killed to a lesser extent. The level of the cross-reaction was comparable with the cross-reaction by effector cells of *nu/+* origin. The specificity of the population reacting with TNP-coupled autologous cells was further characterized by nonradiolabelled target-cell inhibition. The results presented in Fig. 1a show that the population exhibits a strict specificity. The specificity is restricted to both the self-MHC antigens and to the hapten: autologous cells derivatized with 4-hydroxy-5-iodo-3-nitrophenyl (NIP) or allogeneic cells derivatized with TNP did not compete with TNP-derivatized autologous target cells. Cytotoxic T cells generated in secondary cultures showed the same specificity (Fig. 1b). The *nu/nu* effector cells lysing TNP-modified autologous target cells specifically were characterized with respect to the expression of Thy-1 antigen in complement-dependent cytotoxic assay by using an antibody to Thy-1.2 antigen (Table 2). Anti-Thy-1.2 antibody and complement treatment of the effector-cell populations abolished the killer activity demonstrating that these cytotoxic cells were T lymphocytes.

The existence of cytotoxic cells in *nu/nu* mice has been observed previously⁷. The cytotoxic activity was mediated by natural killer (NK) cells, which are known to be Thy-1 negative; their specificity does not show restriction to MHC and their activity is usually determined in a 24-h cytotoxicity assay⁸. The cytotoxic cells induced by TNP-modified autologous cells in the presence of TCGF show all the characteristics of CTLs. They carry Thy-1 antigen, their specificity is restricted to the self-MHC structures and their activity can be determined in a 4-h cytotoxicity assay. The presence of alloreactive pre-effector T cells has been observed in the peripheral lymphoid organs of *nu/nu* mice⁹. These cells were able to differentiate to alloreactive CTLs in the presence of TCGF. Therefore it is supposed that the *nu/nu* mouse is defective in the production of a factor which helps pre-effector T cells to differentiate to immuno-competent, alloreactive, CTL precursor cells. Our experiments show that CTLs with specificity to TNP-modified self-MHC structures are differentiating from *nu/nu* spleen cells after stimulation with hapten-modified autologous cells in the presence of TCGF.

Table 1 The specificity of anti-TNP CTL responses of *nu/+* and *nu/nu* mouse spleen cells

Responder	Stimulator	Target	% Specific ^{51}Cr release at		
			30:1	10:1	3.3:1
<i>nu/+</i>	—	B6-TNP	-6.2	-3.4	-1.0
<i>nu/+</i>	CM	B6-TNP	4.3	-1.2	-1.8
<i>nu/+</i>	B6-TNP	B6	-1.0	-1.0	-3.5
		B6-TNP	61.9	44.6	21.9
		B10 · BR	9.3	3.2	0.6
		B10 · BR-TNP	22.3	8.9	1.6
<i>nu/nu</i>	—	B6-TNP	-3.6	-2.2	0.6
	CM	B6-TNP	-10.8	-4.2	-1.7
	B6-TNP	B6-TNP	-4.8	-4.1	-3.0
	B6-TNP + CM	B6	-3.9	-2.3	-0.3
		B6-TNP	38.5	11.4	5.6
		B10 · BR	3.5	2.1	0.0
		B10 · BR-TNP	15.5	0.5	0.6

The generation and assay of CTLs of *nu/+* origin were carried out as described previously⁴. Briefly, 5×10^7 responding cells and 2×10^7 TNP-derivatized¹² mitomycin C-treated stimulator cells were co-cultured in 25-cm² tissue culture flasks in RPMI 1640 medium containing 10% fetal calf serum (Gibco), supplemented with 10 mM HEPES, 200 mM glutamine, 50 IU ml⁻¹ penicillin, 50 µg ml⁻¹ streptomycin and 5×10^{-5} M 2-mercaptoethanol. After 5 d of co-culture in a humidified CO₂ incubator, the cells were collected and their cytotoxic activity and specificity determined. The target cells were concanavalin A (Con A)-stimulated T-cell blasts labelled with ^{51}Cr ($\text{Na}_2^{51}\text{CrO}_4$; Radiochemical Centre). The attacker (A) and target (T) cells were incubated at different ratios for 4 h, then the supernatants were collected for γ counting. The corrected per cent lysis was calculated according to the following formula:

$$\frac{\text{experimental release} - \text{medium release}}{\text{maximal release} - \text{medium release}} \times 100$$

Medium release was 12–34% of the maximal release. CTLs from *nu/nu* spleens were generated in the same way, except that conditioned medium (CM), containing TCGF, was added to the cultures. The CM was prepared according to the method of Gillis *et al.*¹³, using rat spleen cells (strain SDK).

Table 2 Demonstration of the presence of Thy-1 antigen on cytotoxic cells derived from the spleens of *nu/nu* mice

CTL population	Treatment	% Specific ^{51}Cr release
Primary	Medium	20.8
	Anti-Thy-1,2	26.8
	Anti-Thy-1,2 + C'	0.9
	C'	20.1
Secondary	Medium	31.8
	Anti-Thy-1,2	41.3
	Anti-Thy-1,2 + C'	0.0
	C'	33.7

The antibody-plus-complement (C') treatment of the attacker cells was carried out in the wells of U-bottomed microtitration plates. Attacker to target cell ratio was 20:1 for primary and 5:1 for secondary effector cells. Target cells were TNP-derivatized Con A blasts of B6 mice. Attacker cells were incubated with a monoclonal anti-Thy-1.2 antibody (supernatant F7D5) at the predetermined optimal dilution (1:50) for 30 min at 24°C in RPMI 1640 medium. After incubation with the antibody, the cells were washed twice with medium and rabbit serum was added to the cells as a source of complement (1:8 dilution). The cells were further incubated for 40 min at 37°C, then washed twice. 10^4 target cells were added per well and incubated for another 4 h at 37°C in a CO₂ incubator, then the supernatants were collected for γ counting.

Several hypotheses have been proposed to explain the thymic function in determining the H-2 restriction specificity of CTLs. These can be divided into three categories: (1) the thymus drives differentiation, that is, the high mutation rate of germ-line genes within the thymus, creates the specificities; (2) the thymus

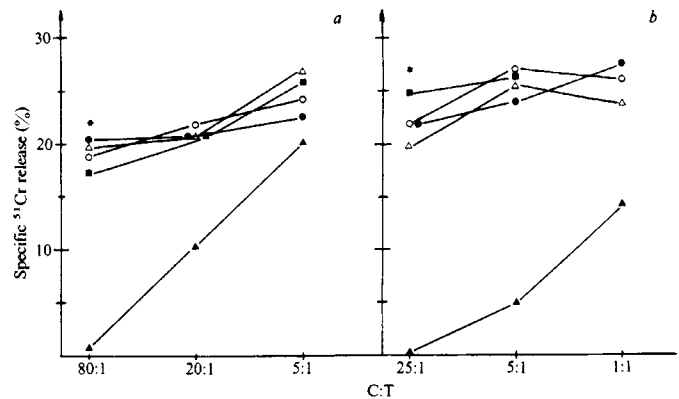


Fig. 1 Specificity of primary (a) and secondary (b) CTL populations of *nu/nu* mice reacting with TNP-derivatized autologous target cells. Secondary CTLs were generated by restimulating 10^5 cells 10 d after *in vitro* priming with 10^5 TNP-derivatized syngeneic lymphocytes in the presence of TCGF. The cells were collected 4 d after restimulation and their cytotoxic activity and specificity were determined. Target cells were TNP-derivatized Con A-induced T-cell blasts of B6 mice. Nonradiolabelled competitor cells were spleen cells, their modification with TNP was carried out as described previously¹⁴. Attacker cells were seeded in 50-µl volume in the wells of U-bottomed microtitre plates, in triplicates. 50 µl of nonradiolabelled competitor (C) and 100 µl radiolabelled target (T) cells were added at 4°C, the cells were spun briefly and incubated for 4 h at 37°C, and the supernatants collected for γ -counting. The attacker to target cell ratio was 20:1 in the primary response and 1:1 in the secondary response. Competitors were: C57Bl/6 (△), C57Bl/6-TNP (▲), B10 · BR (○), B10 · BR-TNP (●) or C57Bl/6-NIP (■) cells. * Marks the level of kill in the absence of competitor cells.

selects cells for further maturation from an already differentiated repertoire; (3) the thymus only influences the maturation of T cells. Our data strongly oppose the first alternative.

The possibility that *nu/nu* mouse spleen cells would not behave in the same way as prothymocytes from normal mice was also considered. The possible residual thymic function in *nu/nu* mice, as well as the maternal (*nu/+*) origin of the precursors, are difficult to exclude. Therefore, we have also used prothymocytes from normal mice (bovine serum albumin gradient purified¹⁰, Thy-1 negative) as responders and the results obtained were similar to those with *nu/nu* spleen cells described above. Hünig and Bevan¹¹ have also reported that the anti-TNP CTL responses of nude mice are H-2 restricted.

I.A. was supported by a scholarship from the Hungarian and Finnish Academies of Sciences. We thank Professor Olli Mäkelä for useful discussions and for critical revision of the manuscript, and Dr E. Simpson for supernatant F7D5.

Received 11 June; accepted 14 November 1980.

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Interferon-neutralizing antibodies in a patient treated with human fibroblast interferon

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During recent years clinical trials have shown that human leukocyte interferon (HuIFN- α) may be useful in the treatment of cancer, but very little has been done concerning the possible use of human fibroblast interferon (HuIFN- β). Treuner *et al.*¹ recently reported the successful treatment of a nasopharyngeal carcinoma with HuIFN- β : in the course of IFN-therapy a HuIFN- β neutralizing activity appeared in the serum of this patient. We report here that such activity is due to IgG antibodies—this study is the first to present evidence for antigenicity of IFN in a homologous system.

The IFN used in therapy was a diploid cell-strain IFN (HuIFN- β) (Rentschler Co., Laupheim) produced in human foreskin fibroblasts (FS-4) by superinduction with poly(I·C), cycloheximide and actinomycin D. The IFN preparations were purified and concentrated using perchloric acid and ammonium sulphate precipitation followed by column chromatography and ultrafiltration. The specific activity of the HuIFN- β preparation used was $1\text{--}2 \times 10^6$ IU per mg protein.

The patient S.R. was treated by i.v. infusions with 4×10^6 units of interferon daily for 5 weeks, followed by three applications per week¹. Repeated analyses over a period of 6 months showed measurable IFN serum levels after injection¹. Subsequent control analyses failed to show IFN activity after i.v. injection of 4×10^6 IU. Therapy was stopped after treatment for 10 months (using a total of $\sim 5 \times 10^8$ units). The serum was analysed for neutralizing activity against HuIFN- β and immunoglobulins were purified. Unfortunately, no earlier serum samples were available.

Serial 2-fold dilutions of serum were incubated for 1 h at 37 °C and 2 h at 4 °C with eight units of either FS-4 HuIFN- β or HuIFN- α . IFN assays were done by plaque reduction assay in RITA cell culture microplates (monkey kidney cell line) using vesicular stomatitis virus (VSV) as challenge. A simultaneous titration of the IFN used was included in each case and the lowest dilution of antiserum examined for direct inhibition of VSV. IFN titres were expressed in terms of HuIFN- β reference standard G-023-902-527 (from NIH, Bethesda), and the neutralizing titre was taken as the highest dilution of serum that inhibited by 50% the protective activity of at least eight units of IFN. The anti-HuIFN- β titre of untreated serum from S.R. was about 10^{-3} using HuIFN- β (Rentschler) or HuIFN- β (NIH) as antigen, or when HuIFN- β (Rentschler) in the serum of an IFN-treated child was used. A neutralization assay in which the concentration of HuIFN- β was varied showed that 50 μ l of serum from patient S.R. were able to neutralize $\sim 10^4$ units of HuIFN- β . Substitution of human serum for fetal calf serum did not induce a change in the anti-HuIFN- β titre. Preincubation of RITA cells with serum from patient S.R. for 20 h inhibited the activity of eight units of HuIFN- β up to a dilution of 1:8—this inhibition could be due to HuIFN- β receptor antibodies or cross-reacting antigens of RITA and FS-4 cells.

Attempts to show neutralizing activity against HuIFN- α in the serum from patient S.R. were unsuccessful². The assay was performed with eight units of HuIFN- α mixed with an appropriate amount of FS-4 cell preparation to rule out the possibility that the presence of antibodies to antigens of the FS-4 cell preparation other than HuIFN- β co-precipitated with IFN to produce a reaction which was erroneously interpreted as HuIFN- β neutralization. No inhibitory effect of serum from patient S.R. against HuIFN- α was seen in these conditions. However, we cannot exclude the possibility that the antibody observed was produced against a complex of HuIFN- β + FS-4 protein, which could be a result of the method of purification.

Serum taken from the patient S.R. 9 months before IFN therapy showed no neutralizing activity against HuIFN- β . As we had no earlier preserum samples we were unable to check the possibility that disturbed immune function might have resulted in production of autoantibodies against endogenous HuIFN- β .

Serum taken from patient S.R. after IFW therapy was heated at 56 °C for 30 min before use then 1 ml was absorbed three times with a total of 6×10^8 FS-4 cells; serum-cell mixtures were incubated with agitation at 37 °C for 30 min and 4 °C for 30 min. Serum was centrifuged at 6,000g for 30 min to remove cells then the total IgG content measured by immunodiffusion with Tri-partigen plates (Behring, Marburg)—this was reduced from

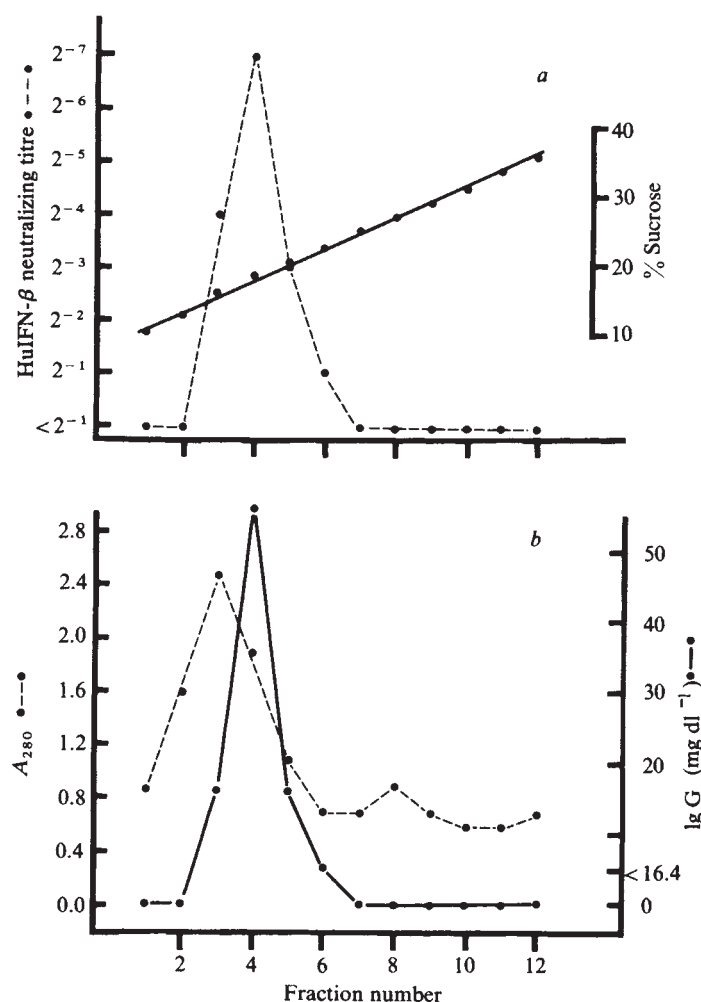


Fig. 1 *a*, Determination of HuIFN- β neutralizing titre. *b*, Total protein content and concentration of IgG after sucrose density-gradient centrifugation of FS-4 absorbed serum from the patient S.R. Serum was centrifuged in a 10–37% sucrose density-gradient for 20 h using a SW-40 rotor (Beckman Instruments, Palo Alto, California) at 154,000g. Fraction numbering was started at the top of the gradient. Determination of anti-HuIFN- β titre (broken line, *a*), IgG concentration (solid line, *b*) and total protein content (A_{280} , broken line, *b*) was done with fractions of the same gradient.

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900 mg dl⁻¹ before absorption to 550 mg dl⁻¹ without diminishing the anti-IFN titre. Preincubation of RITA cells with the absorbed serum did not inhibit HuIFN- β activity.

To demonstrate that the HuIFN- β neutralizing activity was in fact due to antibodies, activity was determined after sucrose density-gradient centrifugation of FS-4 absorbed serum from S.R. Total protein content (E₂₈₀) and concentrations of IgG in sucrose density-gradient fractions of serum are shown in comparison with anti-HuIFN- β titres in Fig. 1. The peak of anti-HuIFN- β activity was found in fraction 4 (Fig. 1a) which correlates with the IgG (Fig. 1b) and the IgA peak (not shown). The IgM peak (fraction 8) showed no measurable HuIFN- β neutralizing activity. In addition, serum IgG from patient S.R. was purified by ion-exchange chromatography to demonstrate that the anti-HuIFN- β activity was due to IgG antibodies. FS-4 cell-absorbed serum was precipitated with saturated ammonium sulphate, pH 6.5, and the precipitate dialysed against 0.02 M phosphate buffer, pH 8.0. IgG was prepared from the precipitate by chromatography on DEAE-Sephacel column (Pharmacia, Uppsala, Sweden) equilibrated with the same buffer. The protein peak passed through with the first buffer volume contained most of the anti-HuIFN- β activity. Immunodiffusion showed that there was no IgA or IgM contamination of the purified IgG. Immunoelectrophoreses of

0.7 mg IgG ml⁻¹ against rabbit anti-human serum protein (Medac, Hamburg) revealed only one component in the IgG preparation.

The first successful demonstration of antiserum to an IFN was reported by Paucker and Cantell³. However, studies on the antigenicity of IFN have shown that they are not antigenic in homologous systems⁴. In a number of clinical trials no neutralizing activity could be demonstrated in the sera of any patients, even after treatment with several millions of human IFN units^{5,6}.

This study presents a strong case for the presence of antibodies to HuIFN- β in the serum of patient S.R. treated successfully with $\sim 5 \times 10^8$ HuIFN- β units. A number of other patients in the Department of Pediatrics in Tübingen were treated with the same preparation of HuIFN- β —three of these had a nasopharyngeal carcinoma⁷. Total remission of the carcinoma after HuIFN- β therapy was shown only in S.R. who was the only patient in which HuIFN- β neutralizing antibodies could be demonstrated, although several others were treated with equally high doses.

We are now attempting to determine whether the patient's cells lack the genes for HuIFN- β and further experiments are being done to clarify this phenomenon of anti-IFN production and its possible clinical significance.

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Received 26 September; accepted 27 November 1980.

Functional reactivation of the deafferented neostriatum by nigral transplants

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Functional deficits following brain lesions can be due not only to the disruption of conduction in specific input and output pathways passing through the site of injury, but also to the loss of important regulatory systems controlling the functional state of neuronal circuitries in areas distant from the lesion. For example, the behavioural disturbances that result from lesions of the nigrostriatal dopamine (DA) pathways^{1–3} can be reversed by administration of dopamine receptor-activating drugs, such as L-dopa or apomorphine^{4–6}. This suggests that the lesioned dopaminergic system, rather than conveying specific input and output signals, is normally acting on neuronal machineries whose activity levels are set by the activity at the dopaminergic synapses. Thus the neurological deficits resulting from these lesions are due to functional inactivation of otherwise intact neostriatal circuitries. Previous studies have shown that intracerebral transplants of embryonic substantia nigra can compensate for drug-induced^{7–9} as well as spontaneous asymmetric motor behaviour¹¹ (expressed as a tendency to move in circles towards the lesioned side), whereas the sensorimotor asymmetry, which is pronounced in rats with a unilateral lesion of the nigrostriatal DA pathway^{4,10}, was unaffected by the transplant¹¹. We report here that restoration of striatal dopaminergic neurotransmission by nigral transplants in animals with bilateral, complete lesions of the nigrostriatal DA pathways can reinstate not only certain aspects of spontaneous motor behaviour, but also sensorimotor orientation and sensory attention on the side of the body contralateral to the graft.

Young female Sprague–Dawley rats (180–200 g at the time of surgery) initially received a unilateral right lesion of the nigrostriatal DA pathway, produced by an intracerebral injection of 8 μ g 6-hydroxydopamine (6-OHDA; Hässle) into the pathway¹². The completeness of the lesion was tested by assessing the intensity of the amphetamine-induced rotational response according to Ungerstedt and Arbuthnott¹³. When injected intraperitoneally with 5 mg per kg metamphetamine 1 week after the 6-OHDA lesion, all the rats included in the study showed a mean of >8 complete 360° turns per min. This turning rate has previously been found to correspond to a reduction in striatal DA of 97% or more⁹.

About 4 weeks after the 6-OHDA lesion the rats received transplants of the substantia nigra region taken from 16–17-day rat embryos (crown to rump length 15–20 mm) as described elsewhere^{7,9}. The transplants were placed on the dorsal surface of the denervated neostriatum, within a suction cavity made through the anterior parietal cortex and the corpus callosum. Eight control rats received the cavity lesion but no transplants. Six months after transplantation, 14 of the transplanted rats which showed complete compensation of their initial amphetamine-induced turning response (Table 1) were selected for further behavioural testing. (The compensation of amphetamine turning has previously been shown to be a good criterion for animals with surviving transplants and with significant dopaminergic re-innervation of the dorsal part of the denervated caudate-putamen⁹. This was confirmed in the final fluorescence histochemical analysis which demonstrated DA-rich surviving transplants and significant DA fibre ingrowth in each case.)

Seven months after the first 6-OHDA lesion, all 22 rats received a second 6-OHDA lesion to the contralateral nigrostriatal DA pathway. They were maintained by intragastric tube-feeding for 8 weeks, giving 25–40 ml per day of a high-energy liquid diet preparation (Vivonex, Vitrum). All surviving rats were finally killed and the brains taken for fluorescence microscopical analysis using the ALFA method of Lorén *et al.*¹⁴. The rats underwent behavioural testing before and after the second lesion, which involved observations on amphetamine-induced and spontaneous asymmetric motor behaviour, arm

Table 1 Behavioural recovery in nigra-transplanted rats

	Unilateral (right) lesion		Bilateral lesion	
	No transplant	Right transplant	No transplant	Right transplant
	(n = 8)	(n = 14)	(n = 8)	(n = 14)
Amphetamine-induced rotation (net ipsilateral turns per min)				
Before transplantation (1 week after 1st lesion)	14.0 ± 2.5	14.0 ± 0.7*	—	—
3 months after 1st lesion	16.3 ± 1.4	2.7 ± 1.0†	—	—
2 months after 2nd lesion	—	—	0.9 ± 1.4	-11.6 ± 0.8†
Spontaneous rotation (probability of left turn)				
Without activation	0.17 ± 0.04	0.32 ± 0.05†	0.57 ± 0.12	0.92 ± 0.04†
Tail-pinch activation	0.24 ± 0.06	0.46 ± 0.05†	0.61 ± 0.11	0.91 ± 0.03†
Arm preference in T-maze (probability of left choice)	0.03 ± 0.02	0.32 ± 0.07†	0.41 ± 0.09	0.79 ± 0.06†
Sensorimotor asymmetry (right-left scores)				
Without activation	3.00 ± 0.82	4.00 ± 0.72*	0.29 ± 0.52	-4.33 ± 0.67†
Tail-pinch activation	4.17 ± 1.05	3.23 ± 0.63*	1.14 ± 0.77	-4.67 ± 0.50†

Amphetamine-induced rotation was recorded in plastic bowls over 60 min after intraperitoneal administration of 5 mg per kg metamphetamin, after the method of Ungerstedt and Arbuthnott¹³. Scores are net ipsilateral (right-left) turns per min. Spontaneous rotation was recorded in the same bowls, and quarter-turns in each direction were recorded over a 3-min period. Ten min later, a paper clip was attached to the rat's tail to provide mild activation, and again quarter-turns were recorded over a 3-min period. Maze testing: rats were tested for arm preference in an unbaited T-maze of conventional design, with and without tail-pinch activation, as described in detail elsewhere¹¹. Each test consisted of two trials separated by a 5–10-min interval. Three tests were carried out, each separated by several days, and the scores for each animal combined as no differences were found between tests. Sensorimotor asymmetry was tested in a battery modified from Marshall and Teitelbaum^{10,20}, and described in detail elsewhere¹¹. Briefly, the orientation to a light pin-prick at different points on the body surface, to touch of the whiskers and snout, and to the odour of an ammonia-soaked swab, were each rated on a three-point scale (0 = absent, 1 = weak, 2 = normal). The asymmetry scores are the difference between total scores on the two sides of the body (right-left). All tests were performed 3–4 months after the initial unilateral lesion and transplantation in the unilateral animals. They all received bilateral lesions at 7 months, and were retested 6–8 weeks later. The amphetamine rotation and sensorimotor tests indicate net (right-left) scores, with positive values denoting bias towards the right (transplant/initial lesion) side. The spontaneous rotation and maze tests indicate the probability of a left turn, scores <0.5 denote a right bias, and >0.5 a left bias. All values are means ± s.e.m. and comparisons by unrelated Student's *t*-test.

* Not significant, $P > 0.01$

† $P < 0.001$.

preference in a T-maze, and asymmetry in sensorimotor responding, as summarized in Table 1.

After the second 6-OHDA lesion both the controls and the transplanted rats became severely akinetic. Both groups of rats also became aphagic and adipsic, and they had to be maintained by intragastric tube-feeding throughout the 8-week test period.

The aknetic state of the animals was partially reversed by tail-pinch activation. When activated in this way, the non-transplanted control rats showed no side bias in their spontaneous turning behaviour and no asymmetric posture. In contrast, the transplanted rats showed a high degree of turning away from the transplant side (towards the left) (right-hand panels in Table 1) and asymmetric posture, with the head, body and tail describing a curve concave on the side of the second lesion. Without tail-pinch activation the transplanted rats showed an equally pronounced bias for direction of turning in the spontaneous rotation test, although the turning rate was much lower. Before the second lesion (left panels in Table 1), the lesioned, non-transplanted controls showed a strong rotational bias towards the lesioned side; this bias was significantly attenuated in the transplanted rats, both with and without tail-pinch activation.

When tested for arm preference in the T-maze, which can be taken as a measure of the relative degree of sensory attention on the two sides of the rat's body, before the second lesion the non-transplanted controls showed a strong bias (97%) for the right arm contralateral to the intact nigrostriatal pathway. This bias was significantly reduced in the transplanted rats. After the second lesion the transplanted rats showed a marked preference (79%) for the left arm, contralateral to the transplant, whereas the bilaterally lesioned controls showed no such asymmetry (Table 1).

When performed before the second lesion, the sensorimotor orientation test demonstrated an equally marked sensory inattention on the left side of the body in both the transplanted and non-transplanted rats. This deficit was unaffected by mild tail-pinch activation. After the second lesion the controls became equally unresponsive on both sides of the body, whereas the transplanted rats showed a marked asymmetry, with significantly greater response towards stimuli applied on the left side of the body, contralateral to the transplant (Table 1). Thus, with respect to both motor asymmetry, choice behaviour in the T-maze and sensorimotor orientation, the bilaterally lesioned rats with unilateral nigral transplants showed a performance similar to that of rats with a unilateral lesion of the nigrostriatal system.

Thus, our results support the conclusion that an ectopic, intracortical nigral transplant, without any external (that is, drug-induced) activation, can induce recovery in certain aspects of motor behaviour, and in sensory attention and sensorimotor orientation specifically on the side contralateral to the transplant, in the spontaneously behaving animal. In contrast, the severe akinesia, aphagia and adipsia of the bilaterally lesioned rats were unaffected.

Figure 1 shows in diagrammatic form a possible model for the mechanism of action of the ectopic nigral transplant, based on the one previously proposed by Stricker and Zigmond². In this model the DA systems regulate the behavioural responsiveness through modulation of an inhibitory control mechanism. In the intact animal the nigrostriatal DA pathway seems to act as a tonic regulatory system which sets the level of activity in the neostriatal machinery. Removal of this dopaminergic regulatory control function results in inhibition of neostriatal function. Reinstatement of dopaminergic neurotransmission by drugs^{4–6},

or—as in our experiments—by transplantation of DA neurones, can thus be interpreted as a reactivation of an inhibited, but otherwise intact, neuronal machinery. If we assume that the nigrostriatal DA system is inhibitory in nature (see ref. 15 for discussion), then the likely action of the nigral transplant would be to disinhibit elements of the nigrostriatal circuitry.

In the model in Fig. 1 the nigral transplant acts by reducing the threshold for behavioural response (R) to diverse sensory inputs (I). An interesting implication of this model is that recovery of responsiveness to sensory stimuli in the transplanted rats can occur without any access of sensory information to the transplant. In the intact animal various types of sensory stimuli have been shown to activate the nigrostriatal DA neurones^{16,17}. However, in the transplanted animal such activation would not be necessary for behavioural recovery provided that the transplanted neurones have a sufficiently high spontaneous activity. Other parallel observations indicate that this indeed is the case. First, in biochemical determinations of DA and its metabolite, dihydroxyphenylacetic acid¹⁸, the turnover of DA in both the transplant and the re-innervated neostriatum has been found to be as high as that of the intact nigrostriatal system. Second,

¹⁴C-2-deoxy-D-glucose autoradiography has revealed a significant glucose uptake in the nigral transplants, amounting to about two-thirds of that of the intact substantia nigra¹⁸, and third, measurements of the rotational response to the DA receptor agonist, apomorphine, in nigra-transplanted rats indicate that re-innervation of the neostriatum by nigral transplants is accompanied by a partial reduction in the supersensitivity of the neostriatal DA receptors¹¹. This observation is compatible with a partial restoration of dopaminergic neurotransmission in the re-innervated neostriatum.

An important feature of the behavioural effects of the nigral transplants was the specificity of the transplant-induced recovery. Thus, in the bilaterally lesioned rats the transplants did not induce a diffuse, partial recovery in all aspects of the DA deficiency syndrome. Rather, the recovery appeared discrete and complete, such that in those behaviours where recovery did occur (motor asymmetry and choice behaviour) the transplanted animals performed as well as an intact rat, whereas in those behaviours where recovery did not occur (akinesia, aphagia and adipsia) they performed as badly as the lesioned control rats. This can be explained if one assumes that different parts of the striatal complex subserve, at least partly, different types of function (see ref. 19). If so, the transplant-induced recovery would depend on which parts of the striatal complex are re-innervated from the transplant. With the present intracortical transplantation site the ingrowing DA fibres are confined to the dorsal parts of the head of the caudate-putamen, whereas the ventral and lateral parts, as well as nucleus accumbens, are left denervated. The possibility that this regional restriction of the transplant-induced dopaminergic re-innervation determines the pattern and specificity of behavioural recovery is now being investigated.

These results demonstrate that intracerebral transplants can be functional not only after pharmacological activation but also in the spontaneously behaving animal, and that ectopic nigral transplants, which probably lack their normal afferent inputs, can nevertheless compensate for the loss of the rat's own substantia nigra, at least in certain types of behaviour. Functional reactivation of denervated central nervous system circuitries by neural transplants should thus offer an interesting approach to the analysis of mechanisms of neurological and behavioural recovery after brain or spinal cord damage as well as in animal models of neurodegenerative disorders, such as Parkinson's disease.

We thank Birgit Haraldsson, Ulla Jarl and Gertrude Stridsberg for technical assistance. The work was supported by grants from the Swedish MRC (04X-3874), the Wiberg and Kock Foundations, and the European Training Programme in Brain and Behaviour Research.

Received 11 August; accepted 4 December 1980.

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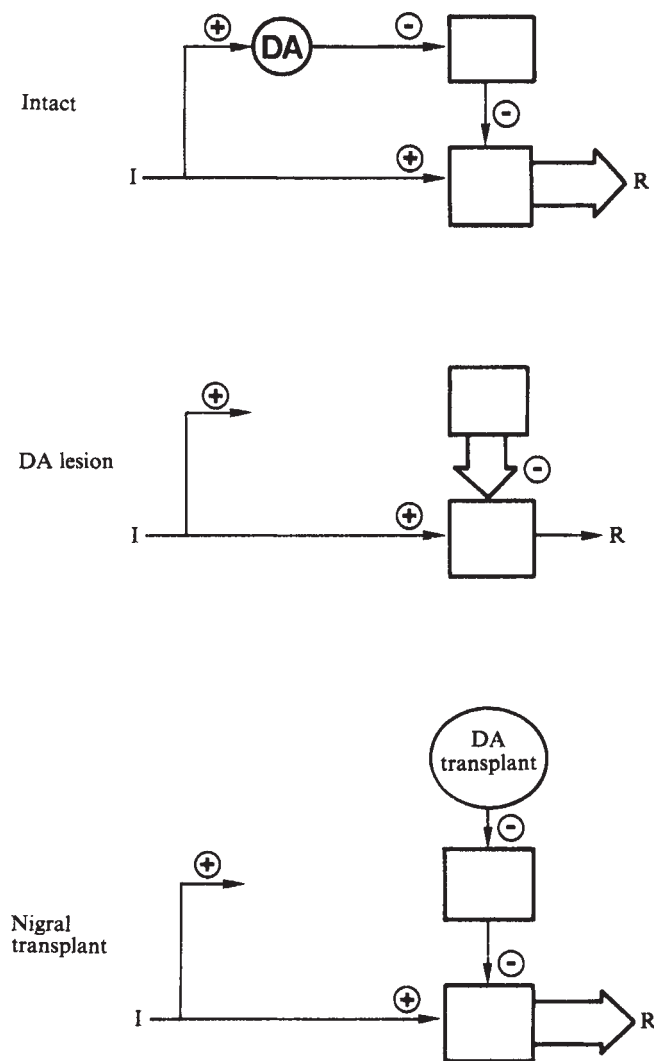


Fig. 1 Proposed mode of action of the DA-producing intracortical nigral transplants in the promotion of behavioural recovery in rats with unilateral or bilateral lesions of the nigrostriatal DA pathway. The behavioural response, R, evoked by an activating sensory input, I, is modulated by the transplant through an inhibitory control mechanism.

Central noradrenergic neurones concentrate ^3H -oestradiol

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Central catecholamines play an important part in the regulation of hormone secretion from the pituitary gland and in the mediation of male and female sexual behaviour¹⁻⁶. Noradrenaline has been shown to stimulate the release of luteinizing hormone (LH) probably by influencing the secretion of LH-releasing hormone (LHRH)^{7,8}. The medial preoptic and hypothalamic areas, including the median eminence, contain noradrenaline-containing terminals which originate from discrete noradrenaline-containing cell groups in the lower brain stem. These cell groups have been identified in the pons and the medulla oblongata by histochemical methods and pharmacological experiments⁹⁻¹⁴. Recent studies with antiserum to dopamine- β -hydroxylase (DBH), the enzyme that converts dopamine to noradrenaline, provided evidence for the existence of noradrenergic cells^{15,16}. These include the locus coeruleus (group A6), a ventrally located more diffuse but continuous subcoeruleus group (group A5), a cell group located dorsal to the nucleus (n.) dorsalis motorius nervi vagi (group A2) and a cell group in or near the nucleus reticularis lateralis (group A1). Using the thaw-mount autoradiographic technique, oestradiol-concentrating neurones have been localized in many areas of the lower brain stem, including the locus coeruleus, n. tractus solitarius, n. dorsalis motorius nervi vagi, and reticular formation¹⁷ where catecholamine-containing neurones exist. We report here the simultaneous localization, in the same histological section, of ^3H -oestradiol and the enzyme dopamine- β -hydroxylase in neurones of the rat lower brain stem with a combined technique of thaw-mount autoradiography and immunohistochemistry^{18,19}, demonstrating that noradrenaline- or adrenaline-containing neurones are oestradiol target cells.

Ten adult female Holtzman Sprague-Dawley rats, castrated 72 h previously, were injected intravenously with [2,4,6,7- ^3H]oestradiol-17 β , specific activity 95 Ci mmol⁻¹, at a dose of 0.5 μg per 100 g body weight and killed after 1 h. Four ovariectomized rats were used to demonstrate the specificity of localization: two each injected with 500 μg of progesterone or 500 μg of 5- α -dihydrotestosterone, 15 min before ^3H -oestradiol. Lower brain stem with pons and medulla oblongata was dissected, placed on a tissue holder and frozen in liquefied propane (-180 °C). Serial frozen sections (4 μm) were cut in a cryostat (Harris), thaw-mounted on photographic emulsion (Kodak NTB3) slides, and exposed in light-proof boxes at -15 °C. The thaw-mount autoradiographic procedure has been described elsewhere²⁰. After 8-12 months of exposure, the slides were fixed for 30 s in 3% paraformaldehyde solution (pH 7.5) at ice temperature, rinsed briefly in 0.01 M phosphate-buffered saline (PBS) solution at pH 7.5, and developed in Kodak D-19 developer (diluted 1:1 in PBS), followed by fixation with Kodak rapid fixer. After photographic processing and subsequent washing with two changes of PBS the autoradiograms were treated with phenylhydrazine-egg albumin to reduce nonspecific staining^{19,21}. After a brief wash with PBS, the autoradiograms were incubated at 4 °C for 48 h with a 1:250 or 1:500 dilution of DBH antiserum²² in PBS containing 1% normal sheep serum and 0.03% Triton X-100. All subsequent steps were carried out at room temperature. After a 3-min wash in PBS, sections were incubated with sheep anti-rabbit γ globulin (1:100 in PBS) for 10 min. After a further 3-min wash with PBS, the autoradiograms were incubated for 20 min with rabbit peroxidase-antiperoxidase complex (1:50).

Another 3-min wash in PBS was followed by immersion of the slides for 10 min in 200 ml Tris buffer (0.05 M, pH 7.6) containing 150 mg of diaminobenzidine tetrahydrochloride and 15 μl of 30% hydrogen peroxide. Autoradiograms incubated with normal rabbit serum were used to test the specificity of the reaction.

Thaw-mount autoradiograms of pons and medulla oblongata stained immunohistochemically with antiserum to DBH showed DBH-positive cells in certain regions of the hind brain (Figs 1, 2). The strongest DBH staining was observed in cells of the locus coeruleus throughout its extensions, both in its dorsal and ventral divisions. About 30-40% of these cells (group A6) showed nuclear concentration of radioactivity after ^3H -oestradiol administration. The localization was specific and probably represented oestradiol because administration of unlabelled oestradiol in excess abolished the nuclear uptake²³, whereas unlabelled progesterone or 5- α -dihydrotestosterone did not. A few DBH-positive cells located at the rostral part near the dorso-lateral corner of the fourth ventricle and at the caudal end of the locus coeruleus were not labelled with ^3H -oestradiol. In addition to the locus coeruleus, positively stained scattered cells were found around the lateral and dorsal aspects of superior olivary nucleus (group A5) and in regions of the lateral lemniscus (group A7). These cells belong to the lateral tegmental area. About 30% of these DBH-positive cells showed nuclear concentration of ^3H -oestradiol. In the ventrolateral medulla oblongata, an accumulation of DBH-positive cells were found in and around the n. lateralis reticularis (group A1)—about 50% of these DBH-positive cells were labelled with ^3H -oestradiol. In the dorsomedial medulla oblongata, DBH-positive cells were located in the n. tractus solitarius near the n.

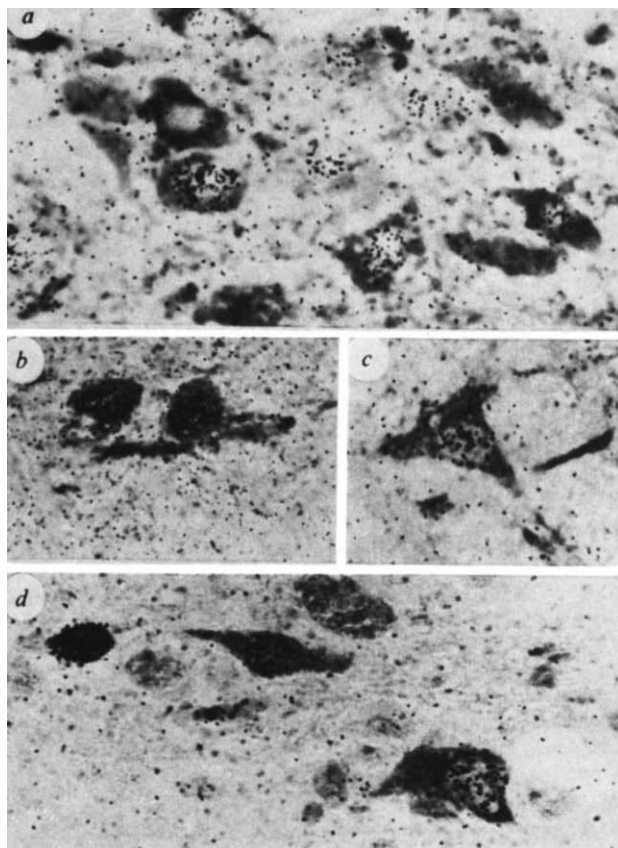


Fig. 1 Thaw-mount autoradiograms of rat lower brain stem 1 h after injection of ^3H -oestradiol, stained by immunoperoxidase method using antiserum to DBH (1:500). Concentration of radioactivity is seen in nuclei of DBH-positive cells in *a*, locus coeruleus, *b*, lateral tegmental area, *c*, n. reticularis lateralis, and *d*, n. tractus solitarius. Exposure time, 360 days; 4 μm . $\times 840$ (*a*, *b*, *c*); $\times 1,050$ (*d*).

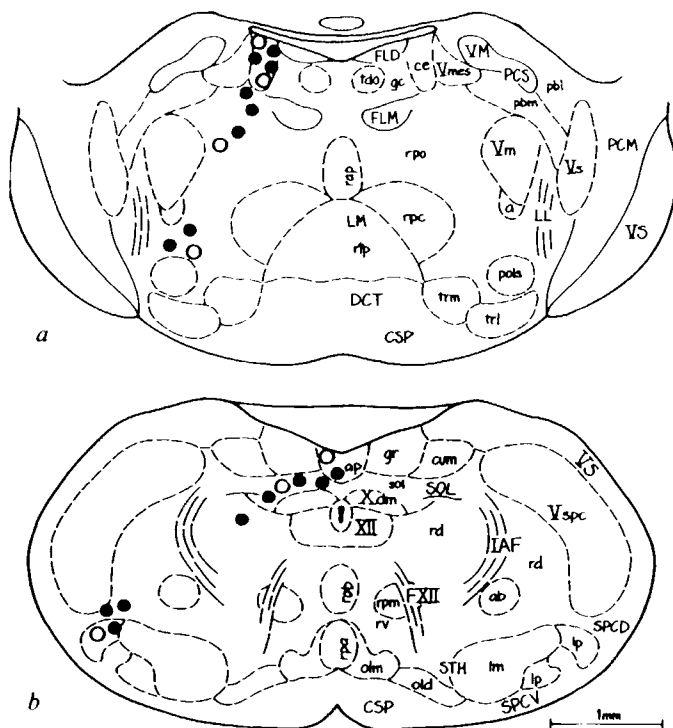


Fig. 2 Schematic drawings of DBH-containing neurones which concentrate ^3H -oestradiol (●) in pons (a) and in medulla oblongata (b). ○, DBH-containing neurones which do not concentrate ^3H -oestradiol. Frontal plane. α , nucleus (n). alpha; ab, n. ambiguus; ap, area postrema; ce, locus coeruleus; CSP, tractus corticospinalis; cum, n. cuneatus medialis; DCT, decussatio corporis trapezoidae; FLD, fasciculus longitudinalis dorsalis; FLM, fasciculus longitudinalis medialis; gc, griseum centrale; gr, n. gracilis; IAF, fibrae arcuatae internae; LL, lemniscus lateralis; LM, lemniscus medialis; lm, n. reticularis lateralis magnocellularis; lp, n. reticularis lateralis parvocellularis; old, n. olivaris accessorius dorsalis; olm, n. olivaris accessorius medialis; pbl, n. parabrachialis lateralis; pbm, n. parabrachialis medialis; PCM, pedunculus cerebellaris medius; PCS, pedunculus cerebellaris superior pars; pld, n. parabrachialis superior; rap, n. raphe pontis; rd, n. reticularis dorsalis medullae oblongata; rpa, n. raphe pallidus; rob, n. raphe obscurus; rpm, n. raphe paramedianus; rpo, n. reticularis pontis oralis; rtp, n. reticularis tegmenti pontis; SOL, tractus solitarius; sol, n. tractus solitarius; SPC, tractus spinocerebellaris; SPCV, tractus spinocerebellaris ventralis; STH, tractus spinothalamicus; tdo, n. tegmentalis dorsalis; trl, n. trapezoides lateralis; trm, n. trapezoides medialis; Vm, n. motorius nervi trigemini; Vmes, n. tractus mesencephali nervi trigemini; VS, tractus spinalis nervi trigemini; Vs, n. sensibilis nervi trigemini; Vspc, n. caudalis tractus spinalis nervi trigemini; Xdm, n. dorsalis motorius nervi vagi; XII, n. nervi hypoglossi.

These results provide direct evidence that DBH-positive neurones in lower brain stem are targets for oestradiol and suggest a direct action of oestradiol on the catecholaminergic neurones. This agrees with the previous observation of formaldehyde-induced fluorescence in oestrogen target neurones in rat lower brainstem²⁴. The combined formaldehyde-induced fluorescence and autoradiography technique, however, does not distinguish between dopaminergic and noradrenergic neurones. Also, there is the possibility of fading of the fluorophore formed from the catecholamine as a result of exposure time of the autoradiograms. The use of antibodies to transmitter-synthesizing enzyme(s) provides a more specific and sensitive approach for the characterization of catecholaminergic neurones. This combined technique is also of great potential for

There is considerable evidence for the effects of gonadal steroids on central catecholamine metabolism. After ovariectomy, administration of oestradiol-17 β results in changes in hypothalamic noradrenaline turnover rates²⁶⁻²⁸, increased tyrosine hydroxylase activity²⁹ and changes on monoamine oxidase activity³⁰. As oestradiol localizes in noradrenergic and, probably, adrenergic neurones, certain effects of gonadal steroids on the regulation of gonadotropin secretion and sexual behaviour are likely to be mediated through direct activation of catecholamine-containing neurones. Results from physiological experiments^{31,32}, although often conflicting, have favoured this hypothesis. For example, noradrenaline systems mediate the stimulatory effects of oestrogen on the release of prolactin^{8,33,34} and of progesterone on release of LH and follicle-stimulating hormone^{7,35}. DBH inhibitors have been shown to provoke anti-ovulatory effect, supporting the stimulating role of noradrenergic neurones in the control of LHRH secretion³⁶. Thus, the positive oestradiol feedback that produces the pre-ovulatory LH surge probably involves activation of the noradrenaline system by a direct effect on catecholaminergic neurones in the n. tractus solitarius and n. reticularis lateralis of the medulla. Day *et al.*³⁷ have used the combined localization of retrogradely transported horseradish peroxidase and catecholamine-fluorescence to demonstrate that the noradrenaline innervation of the medial preoptic area is derived from these nuclear groups. Noradrenaline-containing terminals in the medial preoptic area may stimulate LH secretion by influencing the release of LHRH^{8,35}. The direct association of noradrenaline-containing terminals with the LHRH-producing cells, however, remains to be demonstrated.

This work was supported by PHS grant NS09914 and NIMH grant HD03110. Antiserum was supplied by T. H. Joh.

Received 24 September; accepted 7 November 1980.

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Enkephalins co-exist with oxytocin and vasopressin in nerve terminals of rat neurohypophysis

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Evidence is accumulating that opiates inhibit the release of oxytocin and vasopressin by acting on nerve terminals in the neurohypophysis^{1,2}. Extracts of neurohypophysis have been shown to contain substantial amounts of Met- and Leu-enkephalin^{3,4}, and Leu-enkephalin-immunoreactive (IR) nerve fibres originating in the magnocellular hypothalamic nuclei have been described in the neural lobe, where the two hormones are secreted³. We have compared the distribution of oxytocin, vasopressin and enkephalin immunoreactivity (IR) in the neurohypophysis of the rat, and report here that Met-enkephalin-IR is invariably associated with nerve terminals that contain oxytocin-IR whereas the terminals that contain vasopressin-IR often, but not invariably, are Leu-enkephalin immunoreactive.

Neural lobes of five normal white rats and four Brattleboro rats homozygous for hypothalamic diabetes insipidus were fixed with paraformaldehyde and glutaraldehyde and embedded in Epon. Resin was removed from serial 0.5- μ m sections and Sternberger's unlabelled antibody-enzyme method applied for light microscopic immunocytochemistry⁵ (antibodies are listed in Table 1). Serial ultra-thin and semi-thin sections were cut to examine the fine structure of immunoreactive terminals—the semi-thin section was processed for immunocytochemistry and the ultra-thin section stained with uranium and lead salts for electron microscopy⁶. Cross-reactivity of antibodies was examined in preabsorption controls and in radioimmunoassay (RIA) (Table 1, Fig. 1).

Affinity-purified antibodies against oxytocin and vasopressin recognized two populations of nerve terminals⁷ (Fig. 2*b, c, e*). In their fine structure vasopressin terminals differed from oxytocin terminals by having more densely packed neurosecretory granules. Nerve terminals in neural lobes from Brattleboro rats reacted with oxytocin antibody but not vasopressin antibody.

Preabsorption tests showed that, of the three enkephalin antibodies, only affinity-purified Met-enkephalin antibody I specifically recognized Met-enkephalin (Fig. 1*a, b*). This was confirmed by RIA with several enkephalin analogues and other peptides (Table 1). Met-enkephalin antibody II cross-reacted with Leu-enkephalin (Fig. 1*c, d*) and Leu-enkephalin antibody cross-reacted with Met-enkephalin. This specificity was not affected by addition of Leu-enkephalin or Met-enkephalin in decreasing concentrations or by varying the dilution of the antiserum. Thus, presumably, nerves that were stained by all three antibodies contained Met-enkephalin-like material, and nerves stained by the two cross-reacting antibodies only contained Leu-enkephalin-like material. However, it cannot be ruled out that nerves exhibiting Met-enkephalin-IR contained Leu-enkephalin-like material. Furthermore, it is possible that all the antibodies cross-reacted with larger peptides containing the sequence against which the antibodies were raised—in

preabsorption controls and RIA the Met/Leu-enkephalin antibodies but not the Met-enkephalin antibody I recognized the larger dynorphin⁹.

When enkephalin antibody I, oxytocin and vasopressin antibodies were applied in turn to serial sections, Met-enkephalin-IR nerves were indistinguishable in size, shape and position from most of the oxytocin-IR terminals in a subsequent section (Fig. 2*a, c, f*; Fig. 3), but not from vasopressin-IR terminals (Fig. 2*a, b, g*). Fine structure revealed that the immunoreactive structures were nerve terminals or varicosities filled with electron-dense granules >100 nm in diameter; oxytocin-IR terminals were indistinguishable from Met-enkephalin I-IR terminals. Among terminals with diameters >1 μ m in the neurohypophysis of a Brattleboro rat (Fig. 3), 111 out of 117 Met-enkephalin I-IR terminals coincided with oxytocin-IR terminals in consecutive sections. Six enkephalin-IR terminals without corresponding oxytocin-IR structures were all small and may not have appeared in the next section.

Oxytocin-IR terminals were recognized by Met-enkephalin antibody II (Fig. 2*d, c, h*) and by the Leu-enkephalin antibody which cross-reacted with Met-enkephalin. We therefore assume that oxytocin terminals contain Met-enkephalin-like material. As we had no specific Leu-enkephalin antibody we cannot exclude the possibility that they also contain Leu-enkephalin-like material. In well stained preparations there was nearly one to one correspondence between Met-enkephalin-IR and oxytocin-IR in consecutive sections.

Met-enkephalin antibody II, and the Leu-enkephalin antibody, which cross-reacted with both Leu- and Met-enkephalin respectively, stained certain vasopressin-IR terminals in addition to oxytocin-IR structures (Fig. 2*b-d, h, i*). The Leu/Met-enkephalin antibodies probably recognized Leu-enkephalin-like material but not Met-enkephalin-like material in vasopressin terminals, for the specific Met-enkephalin antibody I did not stain the corresponding structures. However, correspondence

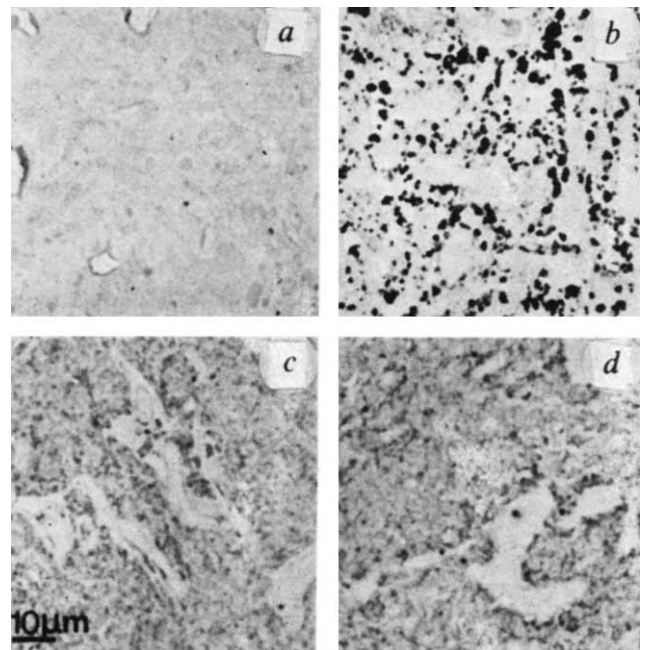


Fig. 1 Preabsorption tests using neural lobe sections with the two Met-enkephalin antibodies. *a, b*, Antibody I; *c, d*, antibody II. *a, c*, Preabsorption by Met-enkephalin; *b, d*, by Leu-enkephalin. When preabsorbed by Met-enkephalin immunoreaction was absent, that is, both antibodies had recognized the antigen and binding sites were blocked. Antibody I, in contrast to antibody II, had not recognized Leu-enkephalin and was reacting with the tissue (*b*). $\times 580$.

Table 1 Antibodies and specificity tests

	Antibodies				Anti-vasopressin (125)
	Anti-Met-enk. I (R26)*	Anti-Met-enk. II†	Anti-Leu-enk.†	Anti-oxytocin (02C)	
Working dilution	1:2,000	1:4,000	1:4,000	1:3,200	1:1,600
Purification by affinity chromatography	+	—	—	+	+
Recognized antigens	Met-enk.	Met-enk. Leu-enk. Dynorphin	Leu-enk. Met-enk. Dynorphin	Oxytocin	Vasopressin α -MSH
Antigens not recognized	Leu-enk. Oxytocin Dynorphin	Oxytocin Arg-vasopressin Lys-vasopressin Vasotocin	Oxytocin Arg-vasopressin	Arg-vasopressin Met-enk. Leu-enk. Neurophysin I/II	Oxytocin Met-enk. Leu-enk. Neurophysin I/II

Antibodies were raised against synthetic peptides. Cross-reactivities with the listed antigens were examined in preabsorption controls by incubating the antibodies for 24 h with the peptides (20 μ l of the antibody solution at half the working dilution + 5 μ g peptide in 5 μ l phosphate-buffered saline (PBS) + 15 μ l PBS). Absence of staining in neural lobe sections after application of the antibody-antigen mixtures indicated that the added peptide had been recognized by the antibody (Fig. 1).

* In a RIA⁸ (using ³H-Met-enkephalin as tracer and unpurified antiserum R 26, 1:16,000), the Met-enkephalin antibody I did not cross-react with the amino acids methionine and leucine, nor the sequences Phe-Met, Phe-NLeu, Phe-Leu, Gly-Gly-Phe-Met, Gly-Gly-Phe-NLeu, and the peptides α -melanocyte-stimulating hormone (α -MSH), β -endorphin, lipotropin hormone (β -LPH), ACTH₁₋₂₄, oxytocin and vasopressin. It cross-reacted less than 0.5% with dynorphin or Leu-enkephalin, but 11% with NLeu-enkephalin.

† It was not possible to isolate a specific population of these antibodies by affinity chromatography. The purification procedures and cross-reactivities of oxytocin and vasopressin antibody have been described elsewhere⁷. The vasopressin antibody stained intermediate lobe cells which indicated cross-reactivity with α -MSH⁷. When preincubated with synthetic α -MSH, staining of intermediate lobe was abolished but staining of neural lobe terminals was not impaired. Application of two α -MSH antibodies⁶ did not reveal α -MSH-IR in the neural lobe.

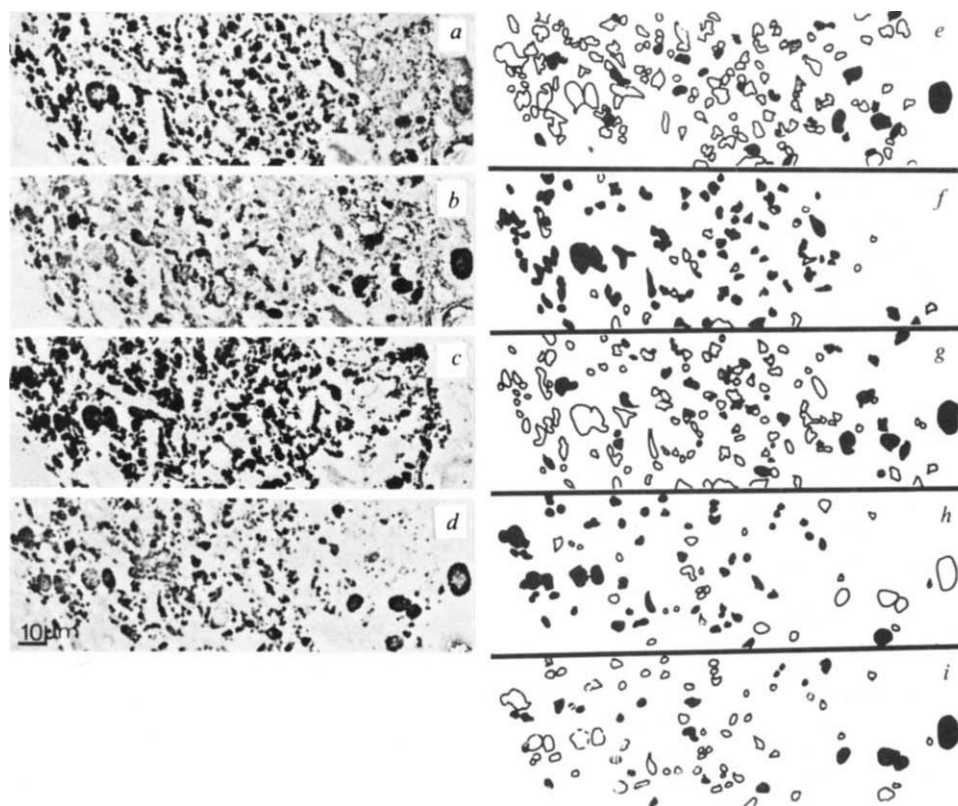
between Leu-enkephalin-IR and vasopressin-IR was less complete than between Met-enkephalin-IR and oxytocin-IR. In many regions vasopressin terminals were not recognized by Met/Leu-enkephalin antibodies. The correlation was better in larger than in smaller terminals (see Fig. 2). In Brattleboro rats in which only oxytocin-IR terminals were found, all these terminals seemed to react with all three enkephalin antibodies. No additional stained structures were observed when the immunoreactivity of the two Met/Leu-enkephalin antibodies was compared with that of the Met-enkephalin antibody I.

Immunoreactive terminals used for comparison had diameters of 1–12 μ m, so that most of them would appear in consecutive 0.5- μ m sections. In one series, 91% of the enke-

phalin-IR terminals (Met-enkephalin antibody II) coincided with either oxytocin or vasopressin terminals; the remaining 9% were all rather small and were presumably cut tangentially. Thus there was no indication of separate enkephalergic nerves.

Our observation of nerves with coexisting Met(Leu)-enkephalin/oxytocin-IR and Leu-enkephalin/vasopressin-IR does not necessarily contradict the report of enkephalin-IR nerves in the rat neurohypophysis³, for that study did not include differential staining with enkephalin, oxytocin and vasopressin antibodies. However, the cell bodies of those enkephalin nerves were located in hypothalamic nuclei that contain oxytocin and vasopressin neurones. In dehydrated rats the decrease in vasopressin and Leu-enkephalin content^{3,10}, but not of thyrotropin-

Fig. 2 Four consecutive 0.5 μ m-thick sections through a neural lobe showing: a, Met-enkephalin I-IR; b, vasopressin-IR; c, oxytocin-IR; d, Met-enkephalin II-IR. $\times 350$. e–i. The immunoreactive structures on two of the sections were compared: e, vasopressin-IR (b) (black spots) compared with oxytocin-IR (c) (contours). The vasopressin antibody recognized other terminals than the oxytocin antibody. f, Met-enkephalin I-IR (a) (contours) compared with oxytocin-IR (c). Coincidence of immunoreactive structures is marked by black filling. The Met-enkephalin antibody I recognized most oxytocin terminals. g, Met-enkephalin I-IR (a) (contours) compared with vasopressin-IR (b) (black spots). The Met-enkephalin antibody I did not recognize vasopressin terminals. h, Met-enkephalin II-IR (d) (contours) compared with oxytocin-IR (c); coincidence marked by black filling. The Met-enkephalin antibody II recognized oxytocin terminals. i, Met-enkephalin II-IR (d) (contours) compared with vasopressin-IR (b); coincidence marked by black filling. The Met-enkephalin antibody II also recognized vasopressin terminals.



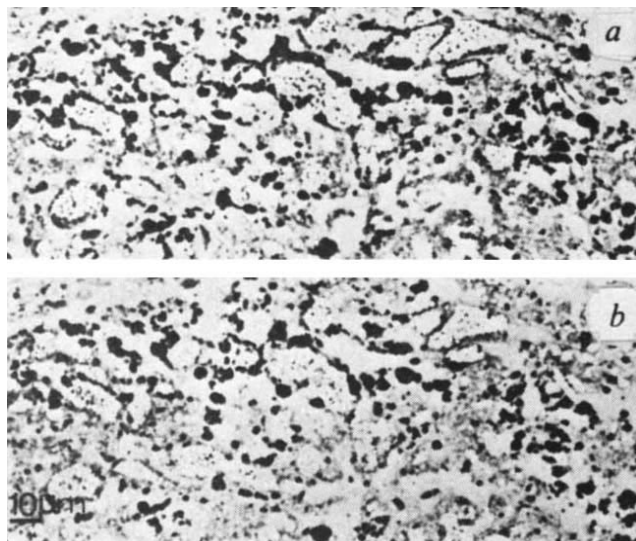


Fig. 3 Two serial sections through a Brattleboro rat neural lobe showing *a*, oxytocin-IR, and *b*, Met-enkephalin I-IR. Most immunoreactive terminals coincide on the two sections. $\times 400$.

releasing hormone or of somatostatin stores³, agrees with coexistence of the two peptides in the same nerves. Also, in homozygous Brattleboro rats enkephalin content was distinctly lower than that in heterozygote littermate controls^{3,10}. Thus it is possible that in this vasopressin-deficient strain a vasopressin-related enkephalin system is impaired.

Our study suggests that enkephalins are co-peptides of the oxytocin and vasopressin neurosecretory systems and that Met(Leu)- and Leu-enkephalin occur in separate neurones. Whether the two peptides are stored in the same secretory granule is not known. The secreted enkephalins may act on autoreceptors on their own nerve fibre or may influence neighbouring terminals, interfering with oxytocin and vasopressin release.

Differential distribution of Met- and Leu-enkephalin-IR has been found in another neuronal system¹¹. However, the existence of separate neurones for the two enkephalins has been questioned by the characterization of a putative common precursor of Met-enkephalin and Leu-enkephalin in the adrenal medulla¹². However, so far only peptides containing either the Met- or Leu-enkephalin sequence have been extracted from brain tissue^{13,14} or pituitaries^{9,15}.

We thank Dr F. W. van Leeuwen for purified oxytocin and vasopressin antibodies. Anti-Met-enkephalin II and anti-Leu-enkephalin were from the Immuno Nuclear Corporation. Anti-Met-enkephalin I was from K.H.V. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 87, B2). K.H.V. is a recipient of a Heisenberg Stipendium.

Received 21 July; accepted 5 November 1980.

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Milk ejection in a marsupial, *Macropus agilis*

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Marsupials give birth to young which are almost embryonic in form, and for the first few months of postnatal life they remain continuously attached to the nipples of the lactating mammary glands. The immature appearance of the neonate and the presence of an elaborate musculature in the wall of the pouch led observers to suggest that the contraction of the pouch functioned to compress the lactating mammary glands and thus force milk into the mouth of the young¹. This view was dismissed when Enders² demonstrated that milk was not expressed in the opossum, *Didelphis virginiana*, following electrical stimulation of the motor nerves supplying the muscles of the pouch, and he implied that milk was obtained solely by the sucking of the young. In most eutherian mammals very little milk can be extracted unless facilitated by an oxytocin-induced contraction of the myoepithelial cells surrounding the alveoli of the mammary gland. It is not known whether such a process of milk ejection exists in a marsupial, although oxytocin has been identified in the posterior pituitary gland^{3,4} and has been used as an aid in the collection of milk⁵. We recorded intramammary pressure from the lactating mammary gland of the agile wallaby, *Macropus agilis*, and examined the pressure responses evoked by injection of oxytocin and electrical stimulation of the presumptive oxytocin neurones. We report here three striking differences from those reported for eutherian mammals: the mammary gland of the wallaby, particularly during the early part of lactation (<100 days), was more sensitive to oxytocin, produced higher intraductal pressures and contracted at lower frequencies of brain stimulation (4 pulses per s). We may therefore conclude that the wallaby, far from discarding during its evolution the milk-ejection response to oxytocin, has refined the response to permit it to feed simultaneously both newly born pouch young (<1 g) and young at foot (>2,500 g).

Studies were made of 18 adult female agile wallabies maintained in the Native Fauna Research Unit of Murdoch University, Western Australia. The agile wallaby is a continuous and opportunistic breeder found abundantly in the wild in the northern parts of Australia and New Guinea^{6,7}. Pregnancy lasts for less than 1 month and the neonate (~650 mg) attaches to one of the four nipples in the pouch and induces a lactation confined to that mammary gland for about 350 d. Lactation is characterized by three stage: (1) pouch young continuously attached to the nipple (0–100 days); (2) pouch young detached from the nipple (80–210 days), and (3) young at foot and sucking occasionally (190–360 days). Evacuation of the pouch occurs at ~200 days—this triggers reactivation in the growth of the embryo in the uterus with the birth of the baby 26 days later⁷. The neonate attaches to one of the three unused nipples and as a result a second lactation develops in parallel to the previous one, but lagging by over 200 days.

After capture, the pouch young were removed and aged⁶, and the mothers (10–15 kg) were anaesthetized with intravenous sodium pentobarbitone (30 mg per kg). Intramammary pressures were recorded from one or more of the 20–30 galactophores in the nipples of the lactating mammary glands. Recordings were made from glands, which according to the age of the dependent young, varied over 38–350 days of lactation.

Injections of oxytocin, either into a catheterized tail vein or external jugular vein, promoted a rise in intramammary pressure after 23–60 s, depending on the dose of oxytocin and the

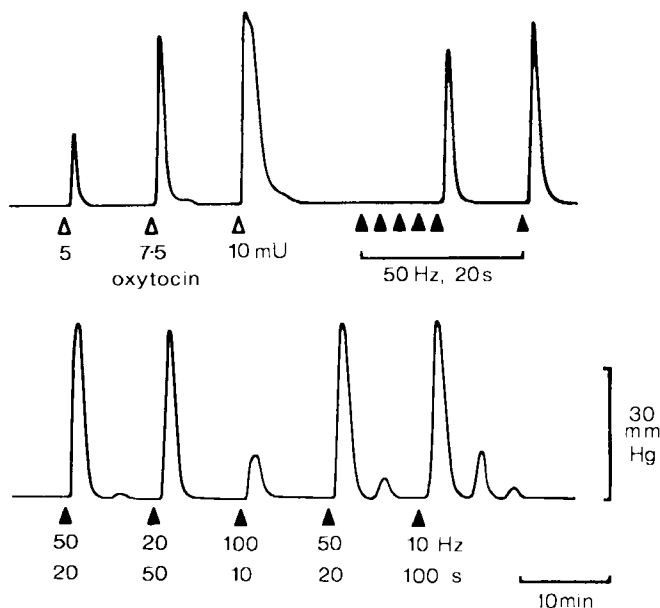


Fig. 1 Recordings of pressure from a galactophore of a mammary gland of an agile wallaby *Macropus agilis* at day 82 of lactation, illustrating the response to injections of oxytocin and electrical stimulation of presumptive oxytocinergic neurones in the region of the basal hypothalamus. The wallaby was anaesthetized with sodium pentobarbitone (30 mg per kg) and the galactophore, one of 27 in the nipple, was cannulated with a 25-gauge needle and connected to a pressure transducer filled with 1% sodium citrate. The two traces are continuous. In the upper trace the first three peaks are the responses to three bolus injections of oxytocin into the external jugular vein. A stimulating electrode was then lowered into the basal part of the hypothalamus from a point of entry in the skull 28 mm anterior to the bregma suture. A 50 Hz $\times$ 20 s pulse train (1 ms $\times$ 700 μ A biphasic pulses) (solid triangles) was applied at intervals as the electrode was lowered, until a position was obtained within the brain at which milk-ejection was induced. The lower trace illustrates a stimulation protocol in which 1,000 pulses were applied at frequencies ranging from 10 to 100 Hz, at 10-min intervals.

ately after birth and, in some species, to allow her intermittently to feed an older sibling on a second lactating mammary gland. Two observations make this a practical possibility: release of oxytocin in response to low-frequency electrical stimulation and the extreme oxytocin sensitivity of the mammary glands in early lactation. In eutherian mammals, the posterior pituitary gland contains a calcium-dependent process of spike facilitation where action potentials entering the nerve terminals in the gland, at ≥ 30 Hz, release 100–1,000 times more oxytocin than impulses arriving at ≤ 10 Hz, and such facilitation is used to generate a pulsatile pattern of hormone release⁹. Frequency facilitation was not observed in the wallaby, which suggests that the process of hormone release differs in some fundamental respect from that in the rat. However, the observation of milk ejection in response to electrical stimulation at 5 Hz raises the possibility that low levels of electrical activity in the oxytocinergic neurones during the early stages of lactation such as might be evoked by the activities of a small pouch young, could result in a small but sustained release of oxytocin sufficient to contract the highly sensitive mammary gland. Such a release would serve in the absence of a tight teat sphincter to produce 'continuous' milk-ejection, with mammary contractions recurring at intervals of ~ 4 min (Figs 1, 2). Furthermore, the pressures generated in these young glands at milk ejection are fivefold higher than those recorded in a rat and are sufficient to cause milk to leak from the nipple in the absence of an attached young. Thus, the original idea that milk was forced into the neonate may not be so far from the truth, although the mechanism is one of milk ejection rather than contraction of the pouch muscles. The decline in oxytocin sensitivity as lactation progresses makes it possible to run two lactations in parallel, but > 200 days out of step. A low level of oxytocin, as discussed above, would not contract a mammary gland feeding a young at foot. Clearly, however, a juvenile of $> 3,000$ g sucking on the enlarged nipple of a mammary gland in late lactation must generate an enormous sensory stimulus compared with that produced by a neonate of < 1 g. In such circumstances it seems plausible that a large release of oxytocin could occur and cause a contraction of the older mammary gland. One consequence, though of little

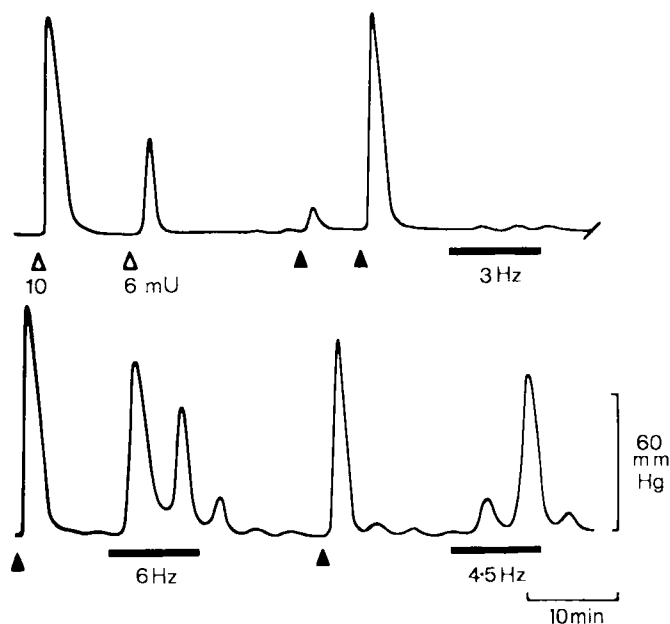


Fig. 2 Intramammary pressure recordings from an agile wallaby at day 116 of lactation. The recording technique and the presentation of the data are as described in Fig. 1 legend. The two traces are continuous, and illustrate the relative effectiveness of prolonged low-frequency stimulation at 3–6 Hz (solid bar) compared with a short burst of high-frequency stimulation (50 Hz $\times$ 20 s) (solid triangles) on the induction of milk ejection. Note that prolonged stimulation produced multiple contractions with pressure peaks at intervals of ~ 4 min.

lactational age of the mammary gland. Peak pressures occurred after 60–90 s and baseline pressures returned after 120–180 s. The threshold dose of oxytocin for the production of a mammary contraction was 2–15 mU, and was substantially lower in recordings from mammary glands in early lactation (< 100 d). The dose-response curve was steep and the pressure responses attained a plateau of 30–60 mm Hg after injections of ≥ 20 –30 mU oxytocin (Figs 1, 2).

Electrical stimulation of the hypothalamo-neurohypophyseal tract in the region of the basal hypothalamus with a 50 Hz $\times$ 20 s pulse train was most effective at promoting milk ejection. The pressure wave evoked by stimulation was slightly more progressive in onset than that produced by a bolus injection of oxytocin, although the peak of the pressure wave was slightly more abrupt. A 50-Hz frequency is optional for the induction of oxytocin release in the rabbit and rat and insufficient oxytocin is released in these species to promote milk ejection at ≤ 20 Hz (refs 8, 9). Surprisingly, when 1,000 pulses were applied to the wallaby in the form of a 10 Hz $\times$ 100 s pulse train the milk-ejection response was equal in amplitude to that seen with higher frequencies of stimulation. Subsequently, very effective increases in pressure were obtained with prolonged stimulation at 4.5 Hz and some evidence of oxytocin release was seen with even lower frequencies (Fig. 2).

Marsupials have evolved a highly successful reproductive strategy when compared with eutherian mammals. A key feature of their success seems to be the refinement of the milk-ejection response to oxytocin to permit the mother to nourish the very immature young continuously in the months immedi-

significance, as the mammary pressure response peaks at 30–60 mm Hg with oxytocin injections >30 mU, is that the older young may give the younger sibling an additional meal.

We acknowledge the financial support of the Australian Research Grants Committee (D.I. 7515759), and the Royal Society for a Commonwealth Bursary.

Received 5 November; accepted 18 November 1980.

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A neuropterous larva uses an allomone to attack termites

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Larvae of *Lomamyia* spp. (Neuroptera: Berothidae) have been tentatively associated with several types of prey since they were first identified¹. In all cases, the assumed prey lived in a confined habitat—an ant nest or termite gallery². *Lomamyia latipennis* Carpenter has been the most thoroughly studied of these unusual Neuroptera. The immature states were described^{3,4} and the association with termites strengthened using *Zootermopsis angusticollis* Hagen (Isoptera: Kalotermitidae) as prey for a group of larvae⁴. A low survival rate of the larvae suggested that another termite might be the normal host. We now report field and laboratory findings which demonstrate the predator–prey relationship between *L. latipennis* and the subterranean termite *Reticulitermes hesperus* Banks (Isoptera: Rhinotermitidae) and describe their unique method of attacking prey with an aggressive allomone.

During the summers of 1979 and 1980 field studies and collections were conducted in Gates Canyon, Solano County, California. Three significant field observations were made concerning the association of *L. latipennis* and *R. hesperus*. On 18 June 1979, a cluster of eight hatched berothid eggs was found hanging on a single stalk from a projection of sapwood on an oak log (*Quercus* sp.) infested with *R. hesperus*. If the larvae behaved like those observed in the laboratory, they crawled up the stalk and across the log surface entering holes and crevices, seeking the termites in the log. When the log was chopped open a well fed first instar berothid larva was found. Then, on 14 June 1980 an adult female *L. latipennis* emerged from a stump of elderberry (*Sambucus coerulea* Raf.) which had been brought into the laboratory about 1 month earlier as a source of *R. hesperus*. No evidence of berothids was found in or near colonies of *Z. angusticollis* or *Zootermopsis nevadensis* Hagen, the only other species of termites recorded in northern California.

Nine female *L. latipennis* were collected under mixed UV/visible light on 2 June 1979 and 9 June 1980. They deposited fertile eggs in the laboratory when fed artificial honeydew consisting of dried yeast (*Kluyveromyces fragilis*), autolysed brewers yeast (*Saccharomyces cerevisiae*) and honey (6 g:1 g:10 g). After hatching, individual larvae were placed in 3-cm³ glass vials half-filled with frass from the termite colonies. Live termites were supplied daily from source colonies maintained in the laboratory. All three species of termite were tested as prey for *L. latipennis* larvae. Only larvae fed *R. hesperus* successfully completed their larval development.

Observations of *L. latipennis* larvae in laboratory colonies of *R. hesperus* indicated that they are true termitophiles, living and moving freely among the termites in their galleries. In the colonies and rearing vials the larvae generally exhibited some caution when near the much more heavily sclerotized termites, but no larva was observed to be attacked. This pattern of coexistence changed only when the larvae were ready to feed.

First instar *L. latipennis* larvae (~0.07 mg), which are much smaller than *R. hesperus* workers (~2.5 mg), displayed a consistent attacking behaviour. A larva repeatedly approached and retreated until the tip of its abdomen was directed at the termite's head. The apex of the abdomen was then lifted and waved past the termite's face, apparently without contact. The termite apparently was not repelled, as it made no obvious effort to escape. One to three minutes later it was incapacitated, lying supine, with its legs moving irregularly. Two to five minutes after the collapse, motion ceased. During this time, the berothid larva continued to advance and withdraw. Feeding occurred only after the prey had become still. If not fed upon, the termite's heart would continue to beat for long as 3 h, showing that the initial immobility was merely paralysis, though death ultimately occurred. The termite may also be preserved by the allomone, but initial tests were inconclusive.

Third instar *L. latipennis* larvae behaved similarly, but the obvious wave of the abdomen was not seen and individuals were observed to kill as many as six *R. hesperus* simultaneously, in the rearing vials. Apparently, the larger larvae produce enough of the allomone to eliminate the need for such precise application to the prey. Second instar larvae are sessile and do not feed⁴. As with the active instars, they were not attacked by the termites, although they were exposed and apparently defenceless.

Soldiers and reproductives of *R. hesperus* were affected in the same manner as the workers. Individual 'pseudoworkers' of the *Zootermopsis* spp. were not affected in this way by *L. latipennis* larvae, although the larvae could feed, with apparent difficulty, on freshly killed *Zootermopsis*. This difference could be due to physiological differences between the termites or simply a matter of dosage, because the *Zootermopsis* spp. are much larger (~8 to >50 mg). If it is a matter of dosage this could explain the partial success of the group rearing using *Z. angusticollis* as prey⁴.

Two compartment test chambers were used to verify that contact between the larva and termite was not required. In each case, a berothid larva and one *R. hesperus* were put into one compartment and a termite into the other. The first type of test chamber was a 0.7-cm³ glass vial divided longitudinally by a strip of filter paper. In 10 tests the second termite was invariably incapacitated within a few minutes after the first had been attacked by the berothid. The second variety of test chamber was a 0.5-cm³ glass tube divided transversely by a disk of cork pierced by a piece of organdy screened capillary tubing. When the prey termite had been attacked, a syringe was used to pull a volume of air, equivalent to that in the first chamber, into the second, as a capillary tube vent permitted air to enter the first. In 7 out of 20 trials, the test termite was also affected. The inconsistent results were attributed to the relatively crude construction of the test chamber and variability in the timing of the suction. In 20 tests without a berothid larva, none of the 40 termites exhibited any signs of distress.

As it can pass through a filter paper barrier and be moved by an air current, the toxin must be active as a gas or an aerosol. This observation of an allomone used in predation is unique among the insects. The only arthropod previously known to use an allomone in this way seems to be the mite, *Womersia strandtmanni* Wharton (Acarina: Leeuwenhoeekiidae)⁵. Because the *L. latipennis* allomone apparently lacks a defensive function, we believe that its primary purpose is aggressive, and we propose the term aggressive allomone to describe this situation. Theoretically, it is possible, and in fact seems probable, that the berothid's allomone evolved from a defensive allomone which was necessary early in their association with *R. hesperus*, before their termitophilia evolved to its current state.

While rearing *L. latipennis* larvae, we noted that Psocoptera (Psocidae) lived in the vials unaffected by the allomone. To test the specificity of the toxin further small Diptera (Drosophilidae) and Hymenoptera (*Coccophagus cowperi* Girault, Aphelinidae and *Metaphycus funicularis* Annecke and *Metaphycus stramineus* Compere, Encyrtidae) were placed in the test chamber. None of these insects, or other berotherid larvae, was affected by the allomone.

Motivated by the unique nature of the allomone, its specificity and the importance of the target insects, efforts are now directed at establishing laboratory cultures of *L. latipennis* and isolating and identifying the active compound(s). Synthesis of the allomone would permit detailed study of its activity and specificity and could yield a valuable new means of controlling termites.

We thank Dr Peter Duelli for his assistance and discussions. This research was supported, in part, by the USDA Western Regional Biological Control Project (W-84).

Received 11 August; accepted 28 November 1980.

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Circulating angiotensin II and adrenal receptors after nephrectomy

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Mineralocorticoid secretion is predominantly controlled by the octapeptide angiotensin II, which exerts trophic actions on the adrenal glomerulosa and acute regulatory effects on aldosterone biosynthesis. The trophic actions include stimulation of angiotensin II receptors and enzymes of the aldosterone biosynthetic pathway^{1,2}, with corresponding enhancement of the aldosterone secretory capacity of the adrenal gland. The positive regulatory action of angiotensin II on its adrenal receptors occurs with elevations of the circulating peptide concentration within the physiological range and probably contributes to the increased sensitivity of the adrenal during sodium deficiency^{3,4}. In this action, angiotensin II differs from other hormones which decrease their target-cell receptors^{5–7}. However, the increase in adrenal angiotensin II receptors following nephrectomy has been interpreted as evidence for a tonic down-regulating effect of angiotensin II on its adrenal receptors⁸. To clarify these conflicting views we evaluated the effects of nephrectomy on adrenal angiotensin II receptors in relation to blood angiotensin II and plasma electrolyte levels. We show here that hyperkalaemia contributes markedly to the post-nephrectomy increase in adrenal angiotensin II receptors, and that circulating angiotensin II levels persist for an unexpectedly long period after nephrectomy, presumably due to tissue generation of the octapeptide.

Adult male Sprague-Dawley rats were bilaterally nephrectomized and groups killed at intervals up to 48 h. Angiotensin II receptors were measured in the zona glomerulosa by binding assay of adrenal capsule homogenate as described previously⁹ and blood samples were obtained for analysis of plasma renin activity (PRA)¹⁰, blood angiotensin II (ref. 11) and serum

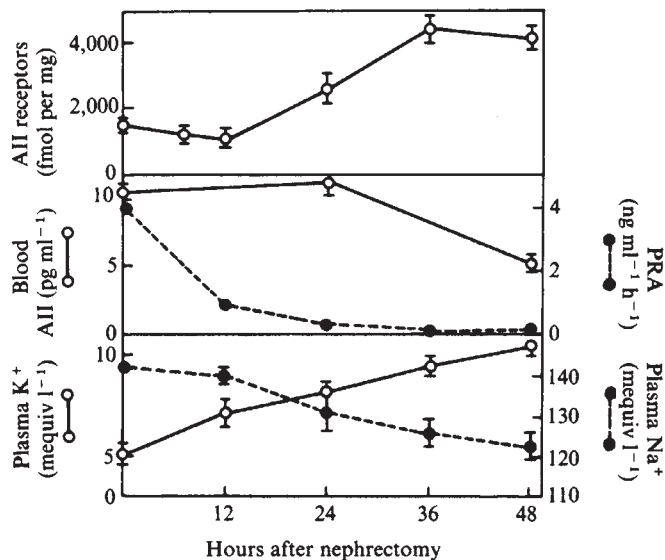


Fig. 1 Effects of nephrectomy on adrenal glomerulosa angiotensin II (A II) receptors (upper panel), plasma renin activity (PRA) and angiotensin II (middle panel) and plasma sodium and potassium (lower panel). The number of angiotensin II receptors was measured in the 300,000g particulate fraction of adrenal capsular tissue⁹, and data points represent the mean and s.e. of three experiments. The data for PRA and blood angiotensin II are the mean and s.e. of assays performed on 6–15 individual animals. Angiotensin II was assayed in 5–7-ml aliquots of blood, collected in acetone and processed as previously described¹¹. The extracts were resuspended in 1 ml of HEPES buffer and angiotensin II concentration was determined by radioimmunoassay (RIA). The ED₅₀ of the RIA was 30–40 pg angiotensin II and the lower limit of sensitivity was less than 5 pg, allowing the detection of values as low as 2 pg ml⁻¹ of blood.

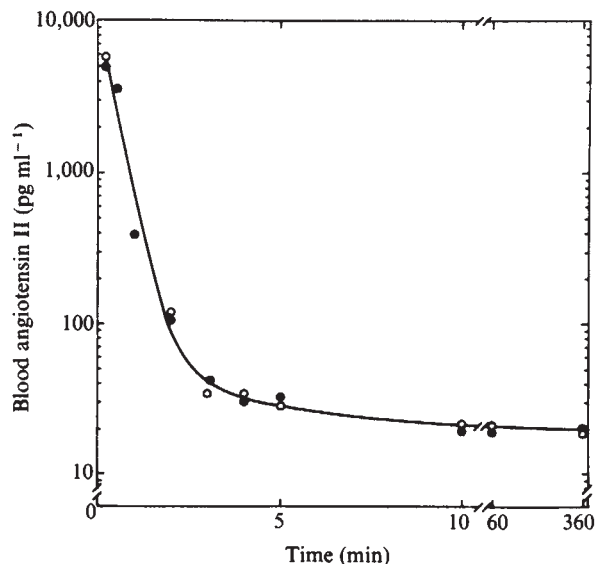


Fig. 2 Rate of disappearance of angiotensin II from the circulation of intact (○) and nephrectomized (●) rats. After injection of 500 ng angiotensin II as a single intravenous injection under ether anaesthesia, animals were killed at timed intervals by decapitation and blood was collected into acetone for extraction and RIA of angiotensin II. Each point represents the mean of values derived from three or four rats.

electrolytes. After nephrectomy, the number of adrenal angiotensin II receptors was unchanged for 12 h, and then increased progressively over the next 24 h to almost double the control level (Fig. 1). As expected, PRA was markedly decreased after nephrectomy and became almost undetectable by 36 h. However, when PRA was reduced by 90% at 24 h, blood angiotensin II levels did not show a commensurate fall, but remained at the levels found in intact rats. Thus, the concentration of blood angiotensin II in 21 nephrectomized rats was $14.7 \pm 6.9 \text{ pg ml}^{-1}$ (mean $\pm$ s.d.) and in 22 normal animals $13.2 \pm 6.0 \text{ pg ml}^{-1}$. Furthermore, after 48 h, when PRA was undetectable, there was only a 50% fall in blood angiotensin II concentration. In contrast, low blood angiotensin II levels were found in sodium-loaded rats ($1.3 \pm 1.5 \text{ pg ml}^{-1}$, $n = 11$) and in animals treated with the converting-enzyme inhibitor, captopril ($2.1 \pm 2.1 \text{ pg ml}^{-1}$, $n = 9$). In nephrectomized rats, plasma sodium decreased, and plasma potassium increased markedly to levels over 10 mequiv l^{-1} within 48 h (Fig. 1). Elevation of the extracellular potassium concentration is known to exert a direct trophic action on the zona glomerulosa, causing marked increases in the number of angiotensin II receptors and aldosterone secretion¹². The rise in plasma potassium following nephrectomy would therefore contribute to the observed increase in angiotensin II receptors in the adrenal gland. These results also demonstrate that the increase in angiotensin II receptors observed after nephrectomy cannot be attributed to the removal of a negative effect of circulating angiotensin II on its adrenal receptors.

The unexpected persistence of normal circulating levels of angiotensin II after nephrectomy was not due to a decrease in the metabolic clearance rate of the peptide in the absence of the kidneys. As shown in Fig. 2, the disappearance of angiotensin II from the circulation after a single injection of the peptide was identical in intact and nephrectomized rats. Computer analysis of the data¹³ gave half times of 0.46 ± 0.1 and $4.0 \pm 0.2 \text{ min}$ for the fast and slow components of the disappearance curves. The rapid clearance of angiotensin II from the circulation indicates that angiotensin formation from non-renal renin or renin-like enzymes must be responsible for the circulating peptide observed in anephric animals.

Such extravascular generation of angiotensin II is also suggested by the finding that several tissue extracts contain immunoreactive angiotensin-like material. The tissue content of angiotensin II in adrenal capsule, decapsulated adrenal, aorta

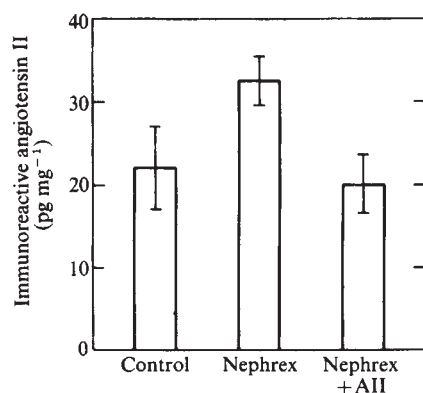


Fig. 3 Immunoreactive angiotensin II in extracts of rat adrenal capsule: effects of nephrectomy and angiotensin II infusion. Angiotensin II was infused at the rate of 200 ng min^{-1} from osmotic minipumps (Alza) implanted intraperitoneally immediately after nephrectomy. Adrenal capsules were rapidly frozen on dry ice, homogenized in 0.01 M acetic acid, boiled for 10 min and extracted in 85% acetone. The extracts were dried down and redissolved in phosphate buffer, and angiotensin II concentration was measured by RIA. Bars represent the mean and s.e. of values obtained for the paired adrenal capsules of 6–10 individual rats.

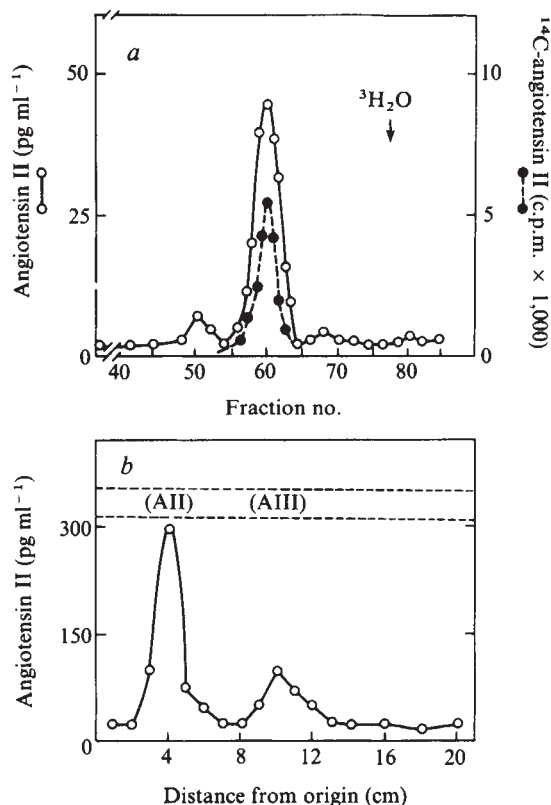


Fig. 4 *a*, Chromatography of adrenal capsule extract on Sephadex G-15 ($1 \times 75 \text{ cm}$) in 0.01 M acetic acid. Angiotensin II concentration was measured by RIA in the individual fractions, and ¹⁴C-angiotensin II was used as a marker in separate experiments to calibrate the column. *b*, Fractionation of immunoreactive angiotensin II by TLC in butanol–3% ammonia (5:1). The positions of angiotensin II and des-Asp¹-angiotensin II are indicated as AII and AIII, respectively.

and uterus was: 22.2 ± 5 , 7.3 ± 2 , 11.5 ± 3 and $14.2 \pm 2 \text{ pg per mg}$, respectively (mean $\pm$ s.e. of 5–10 determinations). In diaphragm extracts, only negligible amounts of the peptide were found. The relatively high content of immunoreactive angiotensin II in the adrenal capsule was not decreased after nephrectomy. The adrenal capsular content of angiotensin II is not solely attributable to receptor-bound peptide, as adrenal angiotensin II was decreased rather than increased after infusion of the peptide (Fig. 3). The absence of increased capsular angiotensin II is not simply the result of receptor down-regulation by the infused peptide⁸, because high doses of angiotensin II reduce but do not abolish its adrenal receptors⁴.

The identity of the angiotensin-like material in adrenal capsules was examined in acetone extracts of tissue homogenates prepared in 0.01 M acetic acid and heated at 100°C for 10 min. Chromatography on Sephadex G-15 revealed a single peak of immunoreactive angiotensin II, corresponding to the established elution profile of ¹⁴C-angiotensin II. TLC of such adrenal extracts revealed two peaks of immunoreactive material, the major component of about 80% corresponding to the R_F of angiotensin II, and a smaller peak corresponding to des-Asp¹-angiotensin II (Fig. 4). The angiotensin II peak was eluted with methanol–ammonia (2:1), dried down and redissolved in phosphate buffer for evaluation of biological activity by adrenal radioligand–receptor assay and by bioassay in collagenase-dispersed adrenal capsular cells¹⁴. The eluted immunoreactive angiotensin displaced ¹²⁵I-angiotensin II from adrenal membranes and stimulated aldosterone production in glomerulosa cells, with equivalent potency to the peptide concentration determined by radioimmunoassay.

The persistence of normal circulating levels of immunoreactive angiotensin II after nephrectomy was observed in assay conditions which detect extremely low blood levels of the peptide after converting-enzyme inhibition⁴ or sodium loading. As plasma renin in addition to injected angiotensin II is rapidly cleared from circulation, generation of angiotensin II in the tissues must account for the presence of the peptide in the blood after nephrectomy. Such local production was supported by the demonstration of angiotensin II in several tissue extracts, including the adrenal capsule. The possible contribution of angiotensinase-dependent hydrolysis of tracer angiotensin II to the apparent presence of the tissue peptide has been noted in kidney extracts^{15,16}. This effect was avoided in the present study by boiling the homogenates for 10 min before acetone extraction. Also, after chromatographic purification, the angiotensin-like material maintained its immunoreactivity and biological activity. The generation of such significant amounts of biologically active angiotensin II in the tissues may account for the normal blood levels of the peptide detected 24 h after nephrectomy. Although decreases in blood angiotensin II levels were previously observed 18 h after nephrectomy in sodium-restricted rats¹², this earlier fall could be related to the higher plasma angiotensinase activity present during sodium deficiency¹⁷.

Renin-like¹⁸⁻²⁰ and converting-enzyme²¹⁻²³ activity are present in several tissues; we have confirmed that renin activity is present in the adrenal capsule and other tissues, and seems to vary with the PRA during altered sodium intake. Such tissue renin could have at least partly accumulated from renal renin in the circulation, as renin activity in the aorta begins to decrease several hours after nephrectomy²⁴. In a related study, PRA fell rapidly after nephrectomy, whereas the blood pressure and the hypotensive response to converting-enzyme inhibition decreased over several hours²⁵. Such a process would also be consistent with the gradual fall of blood angiotensin II observed in the present experiments, with no major decrease until 48 h after nephrectomy. However, tissue isorenin is probably responsible for the local generation of a significant proportion of the angiotensin II that is present in the tissues and continues to circulate after bilateral nephrectomy. Normal levels of renin and angiotensin II have been found in the blood of nephrectomized humans and did not rise after sodium depletion^{26,27}. Such findings, with those presented here, suggest that extra-renal renin may provide a basal level of local and circulating angiotensin II against which the actions of renal renin are superimposed in normal physiological conditions.

Received 2 July; accepted 20 November 1980.

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Gating of a muscle K⁺ channel and its dependence on the permeating ion species

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In excitable cells, ions permeate the cell membrane through ionic channels, some of which open and close in response to changes in the potential difference across the membrane. It has been supposed that this opening and closing (or gating) process is largely independent of the permeating ion. However, we show here that the gating of the resting potassium permeability of frog skeletal muscle depends on the species of ion which carries current across the membrane. The potassium permeability investigated allows K⁺ to move in across the membrane more easily than out^{1,2}. This property is known as inward or anomalous rectification and is shared by cell membranes of skeletal muscle¹⁻³, egg⁴ and certain other cells⁵⁻⁷. In both egg cells⁸ and skeletal muscle fibres⁹, the group IIIB metal ion Tl⁺, which can replace K⁺ in several other systems in experimental conditions¹⁰⁻¹², also permeates the inward rectifier. Indeed, Tl⁺ is more permeant than K⁺ (refs 8, 9). However, when Tl⁺ carries current inwards across the membrane, the inward rectifier inactivates over a brief period when the membrane is hyperpolarized, whereas when K⁺ carries current, the permeability increases with time under hyperpolarization.

Our experiments were carried out on frog sartorius muscle in voltage-clamp conditions¹³ at 1-3 °C, and Fig. 1 summarizes the main result. In muscle fibres immersed in a solution containing 80 mM K⁺, the current flowing under hyperpolarization increases with time (Fig. 1a) along an exponential time course whose time constant falls with increasing hyperpolarization¹⁴⁻¹⁶.

Thus, in K⁺, steady-state currents are larger than initial currents (Fig. 1b), although initial currents themselves yield an inwardly rectifying current-voltage relation¹⁴⁻¹⁶. In the solution we used there was a subsequent slow fall in current caused by K⁺ becoming depleted from the lumen of the transverse tubular system, in whose walls much of the K⁺ permeability resides^{14,17,18}. (The inactivation of K⁺ currents under extreme hyperpolarization, described by Almers^{14,19}, has been shown to be a voltage-dependent block by Na⁺ (ref. 18) and our solutions contained no Na⁺.)

In contrast to the results in K⁺, when muscle fibres are immersed in a solution containing 80 mM Tl⁺ (but no K⁺), the currents which flow under hyperpolarization (Fig. 1c) inactivate along an exponential time course. Thus, steady-state currents are smaller than instantaneous currents, as may be seen from the current-voltage relations of Fig. 1d, and the steady-state current-voltage relation has a region of negative slope.

Steady-state currents, expressed as a fraction of instantaneous currents, are plotted against membrane potential in Fig. 2a. The steady-state inactivation curve may be fitted by a Boltzmann relation of the form:

$$p_{\infty} = \{1 + \exp [-(V - V_p)/a]\}^{-1} \quad (1)$$

where p_{∞} is the fractional current in the steady state at a membrane potential V ; $p = 0.5$ at a membrane potential $V_p = -172$ mV, and $a = 48$ mV.

The time constants for inactivation are plotted against membrane potential in Fig. 2b, showing that the rate of inactivation falls as hyperpolarization is increased, is at its slowest at about -160 to -180 mV, and then increases again under further hyperpolarization. This voltage dependence of the rate of inactivation, and the fact that similar time constants are obtained whether they are measured from the onset of inactivation under a single hyperpolarization or from recovery from inactivation using a two or a three pulse method, suggests that

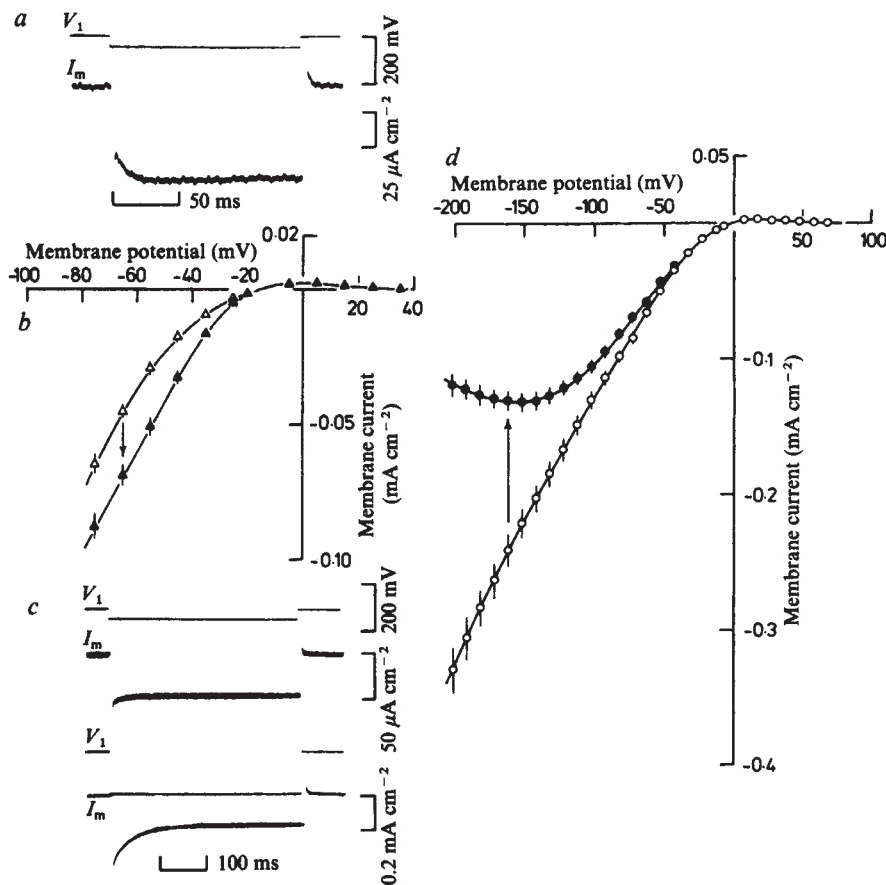
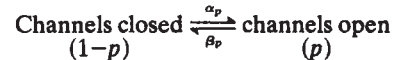


Fig. 1 The gating of inward rectification in K^+ (a, b) and in Tl^+ (c, d) solutions. a, Records of membrane potential (V_1) and membrane current (I_m) from a muscle fibre immersed in a solution containing 40 mM K_2SO_4 (80 mM K^+), 8 mM $CaSO_4$, 113 mM sucrose, 2 mM Tris-maleate buffer (pH 7.2). Fibre resting potential (RP), -16 mV; holding potential (HP), -16 mV; T , 1.2°C, the hyperpolarization is 40 mV in amplitude. b, Current-voltage relations for 12 fibres in 80 mM K^+ solution for instantaneous (Δ) and steady-state ($\bullet$) currents. A least squares method was used to fit the exponential turn-on of currents and to compute instantaneous currents by extrapolation. Symbols and vertical bars give mean $\pm$ s.e.m. of 12 fibres. RP, -14.8 $\pm$ 0.6 mV. Abscissa, membrane potential; ordinate, membrane current. c, Records of V_1 and I_m from a fibre immersed in a solution containing 40 mM Tl_2SO_4 , 8 mM $CaSO_4$, 113 mM sucrose, 2 mM Tris-maleate buffer (pH 7.2). Fibre RP, -2 mV; HP -2 mV; T , 1.2°C, the hyperpolarizations are 40 and 180 mV in amplitude. d, Current voltage relations for fibres in 80 mM Tl^+ solutions for instantaneous ($\circ$) and steady-state ($\bullet$) currents. Steady-state currents were computed by correcting for depletion of Tl^+ by extrapolation to zero time using a least squares fit for a straight line to currents between 250 and 400 ms. Depletion is sufficiently slow to allow such a fit at times <400 ms. Instantaneous currents were computed by making a least squares fit to the exponential decline of currents at short times. Abscissa, membrane potential; ordinate, membrane current. RP, -2.7 $\pm$ 0.4 mV ($n=9$). In both b and d, a linear element, treated as leak, has been subtracted from the current voltage relations. This was obtained by extrapolating from currents obtained with depolarizations greater in amplitude than 60 mV through the holding potential.

the inactivation has simple first order kinetics, which could be represented in the following way:



with p giving the fraction of channels open at a given membrane potential and time, and with the rate constants α_p and β_p approximating to exponential functions of membrane potential (Fig. 2c) of a form expected from equation (1).

The rate of inactivation has a high temperature dependence (Fig. 3a), with the Q_{10} being 2.75 ± 0.14 ($n=6$); at room temperature, the onset of the inactivation cannot easily be separated from the capacity transient accompanying hyperpolarization. The voltage dependence of steady-state inactivation is unaltered by changing temperature (Fig. 3b). The Q_{10} for the amplitude of the Tl^+ currents was 1.42 ± 0.18 ($n=6$), similar to that found for K^+ currents^{14,19}.

The results we describe are very similar to those expected if a blocking cation, present in the external solution, were driven into the membrane by hyperpolarization, blocking the inward movement of Tl^+ . However, our solution contained only Ca^{2+} ,

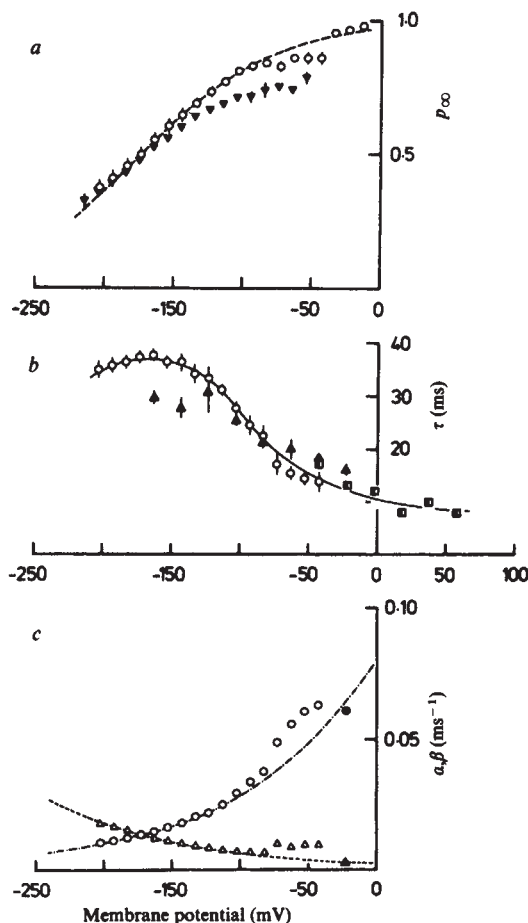


Fig. 2 Inactivation of inward rectification in Tl^+ solutions. a, Steady-state inactivation for 80 mM Tl^+ ($\circ$) and 40 mM Tl^+ + 40 mM K^+ (∇) solutions. Abscissa, membrane potential; ordinate, p_∞ , the steady-state current expressed as a fraction of the instantaneous current. Dashed line, Boltzmann relation given in equation (1) of the text. b, Time constants for inactivation (ordinate) plotted against membrane potential (abscissa). $\circ$, Values obtained from inactivation of currents under hyperpolarization (mean $\pm$ s.e.m., $n=9$); Δ , values obtained from recovery of current at the given membrane potentials after a 180 mV hyperpolarization of 150 ms duration (mean $\pm$ s.e.m., $n=4$); $\square$, rate of recovery at given membrane potential obtained by applying a hyperpolarizing test pulse of 180 mV amplitude and 150 ms duration a variable interval after an inactivating pulse of 180 mV amplitude and 150 ms duration ($n=1$). The line to the points was drawn by eye. $T=1-3^\circ C$. c, Rate constants for the inactivation. $\circ$, $\bullet$: α_p ; Δ , $\triangle$: β_p . Open symbols computed from the open symbols of a and b. Closed symbols, computed from Δ for a 20-mV hyperpolarization in b and the relevant open symbol in a. Dashed and dotted line, $\alpha_p = \bar{\alpha}_p \exp[(V - V_p)/2a]$; dashed line, $\beta_p = \bar{\beta}_p \exp[-(V - V_p)/2a]$ with $\bar{\alpha}_p = \bar{\beta}_p = 1.34 \times 10^{-2} \text{ ms}^{-1}$; $V_p = -172 \text{ mV}$; $a = 48 \text{ mV}$.

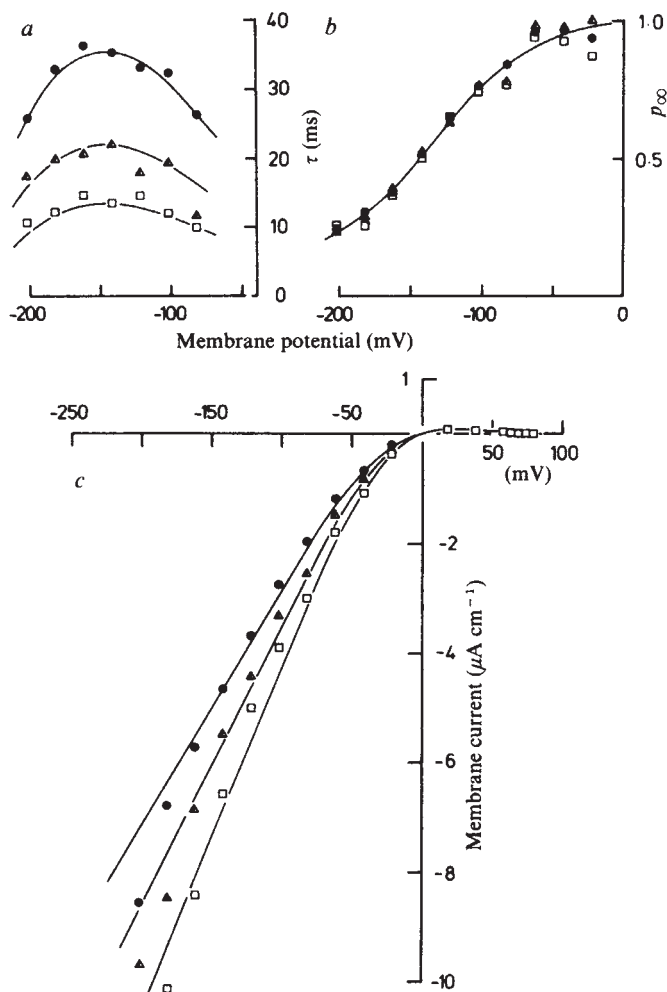


Fig. 3 Q_{10} for inactivation in Tl^+ solutions. *a*, Time constants for inactivation (ordinate) against membrane potential (abscissa), measured at 1.5 °C (●), 5.7 °C (▲) and 10.3 °C (□). The line to the filled symbols (●) was drawn by eye; the others were drawn assuming that the rate of inactivation has a Q_{10} of 3.05 in this fibre. *RP*, -2 mV; *HP*, -2 mV. *b*, Relation between p_{∞} (ordinate) and membrane potential (abscissa) at the three temperatures as in *a*. *c*, Relation between membrane current per unit length (ordinate) and membrane potential (abscissa) for the temperature given in *a*. The line to the open symbols (□) was drawn by eye. Those to the other symbols assume a Q_{10} = 1.57 for this fibre. Currents per unit length were computed using measured values of r_i (Ωcm^{-1} ; sarcoplasmic resistance per unit length) from cable analysis of the response to a 5-mV hyperpolarization (see ref. 13).

apart from Tl^+ , and the effect is unlikely to be a block by Ca^{2+} because: its replacement by Mg^{2+} does not alter the inactivation; Ca^{2+} does not block in K^+ solutions²⁰; those alkali metal ions (Ba^{2+} , Sr^{2+}) which do block inward rectification do so with an effective valency of 1.4, (ref. 20) not 0.5 ($=eF/RT$) as implied for this inactivation by equation (1).

The inactivation cannot be caused by depletion of Tl^+ from the lumen of the T-system. Depletion would not occur at such a high rate¹⁸, its rate would not have such a high Q_{10} (ref. 19 and Fig. 3*a*), the rate would not fall with increasing hyperpolarization^{17,19}, and depletion would not give a steady-state current-voltage relation with a region of negative slope^{19,21}. The same arguments also exclude the possibility that local intracellular accumulation reduces the driving force on Tl^+ . In addition, the steady-state inactivation (Fig. 2*a*) and its rate of onset are little altered in solutions containing 40 mM Tl^+ and 40 mM K^+ .

The mechanism of the inactivation we describe is unclear. It is possible that Tl^+ blocks its own inward movement, but such a self-block seems unlikely to account for inactivation time constants as high as 30–40 ms (Fig. 2*b*). An interaction, within the channel, between K^+ from the internal solution and Tl^+ from outside might also be responsible. An alternative is that Tl^+ (but not K^+) stabilizes a conformational change produced by hyper-

polarization, which results in closure of the channel. The high Q_{10} for the rate at which inactivation occurs may favour this alternative.

Whatever the mechanism underlying inactivation in Tl^+ solutions, the property we describe—dependence of the gating of an ionic channel on the permeating ionic species—may not be unique to inward rectification. It has recently been shown that the inactivation of the Ca^{2+} channels of certain cells^{22–25} depends on the ion (Ca^{2+} , rather than Sr^{2+} or Ba^{2+}) moving through the channels.

We thank Dr N. B. Standen for helpful discussion and critically reading the manuscript, Mr P. Mallory for technical help, the MRC for a fellowship to F.M.A. and the Royal Society for an equipment grant to P.R.S.

Received 15 September; accepted 14 November 1980.

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Cell-free synthesis and processing of multiple precursors to glucagon

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Glucagon, a polypeptide hormone of 29 amino acids, is synthesized in the islets of Langerhans and immunoreactive forms of the molecule have been found in several tissues¹. Like many other polypeptide hormones, glucagon is synthesized via a larger precursor molecule, proglucagon^{2–7}; however, estimates of its size vary considerably and the biosynthetic relationship between some of the putative precursors and authentic secreted glucagon is unclear. Consequently, it was of interest to investigate the primary translation product of glucagon mRNA to relate its size to that of previously described glucagon precursors^{5,6}. Here we provide evidence for three distinct immunoreactive preproglucagon molecules, two of which have an apparent molecular weight (MW) of ~14,000 (14K), the third with a MW of ~16,000 (16K). Furthermore, when microsomal membranes were present during translation, the nascent 14K preproglucagon polypeptides were processed to proglucagon with a higher apparent MW of 15,000. In contrast, the nascent 16K preproglucagon was co-translationally processed to a slightly smaller polypeptide. The data indicate that the 14K and 16K preproglucagons undergo different types of post-translational modification.

When poly(A)-containing mRNA isolated from angler fish islets of Langerhans⁸ was translated in the wheat-germ cell-free protein synthesizing system, four major translation products were identified^{8–10} (Fig. 1, lane 2). Treatment of the products synthesized in the absence of microsomal membranes with a

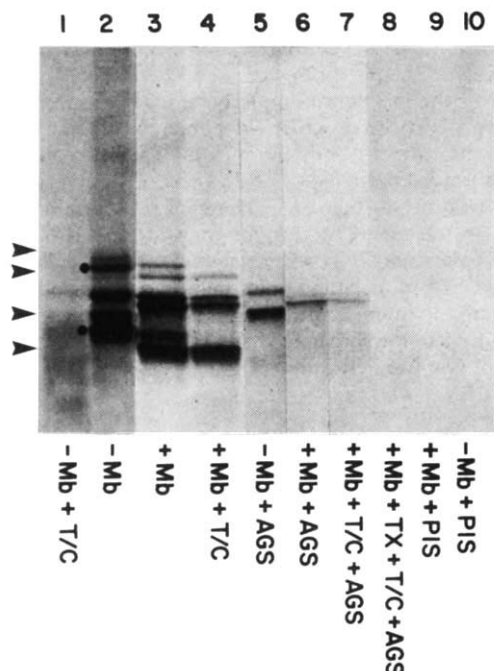


Fig. 1 Synthesis, processing and segregation of proglucagon precursors. Resolution of the translation products was by SDS-polyacrylamide gel electrophoresis using gradients of 12–20% acrylamide⁸; the autoradiograph of the dried gel is shown. Islet mRNA (1 A_{260} unit per ml) was translated in the wheat-germ system, containing 300 $\mu\text{Ci ml}^{-1}$ of ^{35}S -methionine, in the absence (–Mb) or presence (+Mb) of dog pancreas microsomal membranes at 4 A_{260} units per ml (ref. 21). Incorporation into acid-precipitable radioactivity was 100,000 and 55,000 $\mu\text{Ci ml}^{-1}$ in the absence and presence of membranes, respectively. After 60 min incubation at 26 °C, aliquots of the translation products were prepared for electrophoresis directly (lanes 2, 3) and the remainder was subjected to various post-translational assays. Segregation (assayed by resistance to proteolysis), lanes 1, 4, 7, 8: aliquots were adjusted to 5 mM CaCl_2 followed by treatment with a mixture of 250 μg each of trypsin and chymotrypsin per ml (+T/C) for 90 min at 4 °C. Immunoreactive translation products. Lanes 5–8: samples were boiled in 1% SDS then 4 vol of solution A (ref. 22) were added followed by 5 μl of rabbit anti-bovine glucagon serum (+AGS). After incubation for 16 h at 4 °C, the immunoprecipitates were processed for gel electrophoresis²². Lane 7: proteolysis, before immunoprecipitation, was terminated by treatment with 1,000 units of Trasylol and 2 mM phenylmethylsulphonyl fluoride; the samples were then treated with SDS followed by solution A²² and 5 μl anti-glucagon serum. Lane 8: treatment was as for lane 7 except that proteolysis was performed in the presence of 1% Triton X-100 (+TX). Lanes 9, 10: translation products treated with 5 μl of preimmune rabbit serum (+PIS). Upper and lower dots represent the migration of pre-prosomatostatin and preproinsulin, respectively. Arrows indicate the mobility of soybean trypsin inhibitor, sperm whale myoglobin, egg white lysozyme and bovine proinsulin representing MWs 21,400, 17,000, 14,300 and 8,700 respectively.

specific rabbit antiserum raised against bovine glucagon, resulted in precipitation of three polypeptides (Fig. 1, lane 5; Fig. 2). Two of the immunoprecipitates, of apparent MW 14,000, were the most prominent and seemed to migrate as a doublet on SDS-polyacrylamide gels. The third immunoreactive polypeptide, which was less abundant than the 14K precursors, had an apparent MW of 16,000. Identical results were obtained using purified monospecific glucagon antibodies (data not shown). As expected, treatment of the translation products with preimmune serum (lane 10) did not result in precipitation of any detectable polypeptides. The three glucagon-immunoreactive translation products have been tentatively designated preglucagons.

Translation of the islet mRNA in the wheat-germ system supplemented with dog pancreas microsomal membranes, resulted in the synthesis of several additional polypeptides (Fig. 1, lane 3) two of which have been identified as proinsulin⁸ and prosomatostatin⁹. Treatment of these cell-free products with anti-glucagon serum resulted in precipitation of two polypeptides (lane 6). The 16K preproglucagon seemed to have been co-translationally processed to a slightly smaller polypeptide which migrated marginally faster than the precursor. In contrast,

Table 1 Relative efficiency of processing and segregation of proglucagons, prosomatostatin and proinsulin

Prehormone	Prepro-hormone	Processed pro-hormone	Segregated pro-hormone	% Pro-hormone segregated	% Total pre-hormone segregated
Insulin	8,330	5,250 (63%)	4,413	84	53
Somatostatin	2,630	1,150 (44%)	675	59	26
Glucagon, 14K	2,070	1,990 (96%)	1,360	68	66
Glucagon, 16K	2,360	2,390 (100%)	1,330	56	56

The indicated polypeptides were located by autoradiography, excised from a dried gel similar to that shown in Fig. 1 and the radioactivity (c.p.m.) determined in each gel band¹⁰. Segregated prohormones are those polypeptides that were resistant to post-translational proteolysis by a mixture of trypsin and chymotrypsin (Fig. 1). Values in parentheses represent the percentage of the total prepro-hormone processed to the polypeptide. Note that the radioactivity in the processed 16K proglucagon may be due in part to several unidentified polypeptides in addition to glucagon precursors.

the microsomal membranes processed both 14K preproglucagons to a higher molecular weight form, ~15K, as judged by the relative intensities of the immunoprecipitates (Fig. 1, lanes 6, 7). Following processing, we were unable to resolve each of these segregated proglucagon molecules as separate bands, and conclude that they have an identical increase in MW. The processed proglucagon molecules were segregated into the heterologous microsomal membrane vesicles, as evinced by their resistance to post-translational proteolysis (lane 7), and consistent with this observation was the finding that proteolysis in the presence of detergent resulted in complete degradation of the segregated proglucagon molecules (lane 8). We conclude that all three nascent proglucagon polypeptides were segregated into the microsomal vesicles but underwent different post-translational processing. The relative efficiency of processing and segregation of the nascent proglucagons as well as proinsulin and prosomatostatin is summarized in Table 1.

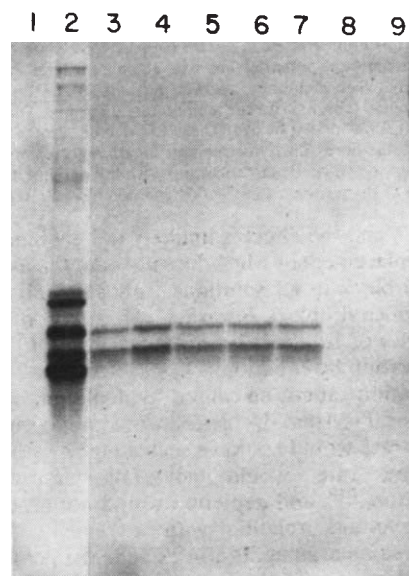


Fig. 2 Immunological characterization of the cell-free translation products having glucagon-like immunoreactivity: competition between unlabelled glucagon and the three putative preproglucagons for binding by anti-glucagon antibodies. Samples of ~300,000 c.p.m. of ^{35}S -methionine-labelled translation products were processed for antibody precipitation as described elsewhere²², using the following conditions. Lane 1: 5 μl of preimmune serum; lane 2: control total translation products; lane 3: 5 μl of anti-glucagon serum; lanes 4–9: samples were treated with 5 μl anti-glucagon serum in the presence of 0.01 ng, 0.1 ng, 1 ng, 10 ng, 100 ng and 1 μg of unlabelled glucagon, respectively. The immunoprecipitates were processed for gel electrophoresis and autoradiography as outlined in Fig. 1. legend.

In the absence of purified glucagon from angler fish and because the amino acid sequence of angler fish glucagon has not been determined, we could not use heterologous glucagon in peptide mapping experiments to identify the translation products. Instead, we used antibody competition experiments to characterize the glucagon immunoreactive polypeptides further. Increasing concentrations of unlabelled glucagon were added to the cell-free translation products, which were then treated with the rabbit anti-glucagon serum (Fig. 2). With increasing concentrations of unlabelled glucagon there was a diminution in the binding of antibodies to all three preproglucagon polypeptides. Furthermore, all three precursors exhibited parallel responses to competition with unlabelled glucagon (Fig. 3). At the higher concentrations of unlabelled glucagon, antibody binding to all three glucagon precursors was abolished (Fig. 3). Most significantly, the antibody displacement curves of the 14K preproglucagons and the 16K precursor were identical to each other and to that obtained for radioimmunoassay of mature secreted bovine glucagon. These results suggest that the same glucagon antigenic determinants are recognized in all three glucagon precursors. It might be argued that these three molecules were immunoreactive because the antiserum was not specific for glucagon, and contained antibodies against other islet polypeptides. Several lines of evidence make this unlikely. (1) All three cell-free antigens manifested the same displacement curves during antibody competition; it is unlikely that three totally unrelated antigens would exhibit the same competition curves. (2) There was no detectable cross-reactivity of our antiserum with bovine pancreatic polypeptide, neither were the major translation products preproinsulin or preprosomatostatin, immunoprecipitable (Fig. 1). Nevertheless, to show unequivocally that all three precursors contain the glucagon amino acid sequence, we are now purifying angler fish glucagon for use in peptide mapping studies.

Three putative preproglucagon molecules are evident on translation of angler fish islet mRNA in the wheat-germ system, and similar results were obtained using the rabbit reticulocyte cell-free system. Two of the precursor molecules (MW ~14,000) are closely related, at least with respect to having similar migrations on SDS-polyacrylamide gels. In addition, these molecules have the expected molecular weight for proglucagon of 12,000 (ref. 5), possessing an NH₂-terminal signal peptide of 20–30 amino acids^{8,9,11}. The third preproglucagon, of MW 16,000, migrated quite separately from the 14K precursors on SDS-gel electrophoresis. The finding of multiple preproglucagon molecules is not without precedent, for two forms of islet preprosomatostatin have recently been described^{10,12,13} and two distinct somatostatin molecules have been isolated from catfish islets¹⁴ and sheep hypothalamus¹⁵.

All the glucagon precursor molecules are somewhat smaller than predicted by Patzelt *et al.*⁶; they found a single proglucagon molecule of MW about 18,000 during pulse-chase experiments using isolated rat islets. The discrepancies in molecular weight may be explained by differences in the gel electrophoresis systems. As the preproglucagon molecules are about four or five times larger than the secreted hormone, it is possible that they contain additional hormone amino acid sequences. It would be of interest to determine if the glicentin amino acid sequence is present in any of the putative preproglucagon molecules. Glicentin, which is a 100-amino acid polypeptide isolated from intestinal mucosa¹⁶, has glucagon immunoreactivity and was recently shown¹⁷ to contain the entire sequence of glucagon.

The most intriguing result of these experiments is that the various nascent preproglucagon molecules undergo different post-translational modifications. The 14K preproglucagons exhibit an apparent increase in MW on SDS-gel electrophoresis when the islet mRNA is translated in the presence of microsomal membranes. Similar observations were made by Patzelt *et al.*⁶ in their studies on rat islet proglucagon. By analogy to other secretory and membrane glycoproteins^{18,19}, this result is consistent with the co-translational removal of a signal peptide and the addition of core-sugars to the growing nascent glycopeptide. In

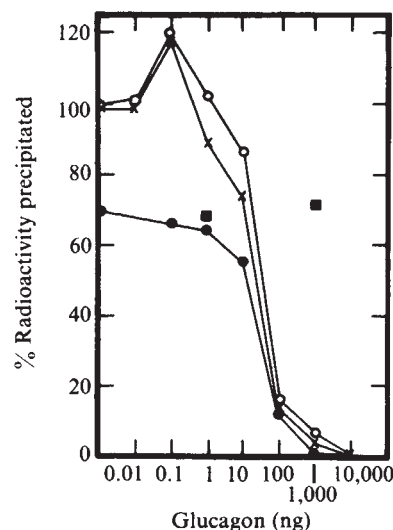


Fig. 3 Quantification of the competition between unlabelled glucagon and preproglucagons for binding by anti-glucagon antibodies. Incubations and treatments were as outlined in Figs 1 and 2 legends. Post-translational incubation with rabbit anti-glucagon serum in the presence of increasing amounts of unlabelled glucagon was as indicated. Control samples received 5 μ l of preimmune serum. The bands corresponding to the 16K and both 14K preproglucagons (not separately resolved) were located by autoradiography, excised from the dried gel and solubilized¹⁰ and the radioactivity determined by liquid scintillation counting. 100% precipitation was equivalent to 1,100 c.p.m. (16K precursor $\circ$ — $\circ$) and 2,400 c.p.m. (14K preproglucagons $\times$ — $\times$). Background precipitation determined with pre-immune serum was 199 c.p.m. Results were calculated after background subtraction. For radioimmunoassay of the anti-glucagon serum ($\bullet$), aliquots of ¹²⁵I-glucagon containing 75,000 c.p.m. of trichloroacetic acid-precipitable radioactivity (~0.75 ng glucagon) were treated exactly the same as the translation products²² and the indicated concentrations of unlabelled glucagon were added followed by 2 μ l of anti-glucagon serum. The antibody-antigen complex was precipitated as described previously⁹ and the radioactivity determined in a γ counter. $\blacksquare$, Competition between ¹²⁵I-glucagon and pancreatic polypeptide (10 ng and 1 μ g).

preliminary experiments we were unable to demonstrate binding of these processed polypeptides to several different lectin affinity columns. Furthermore, there is no evidence that mature glucagon is a glycoprotein, although this does not exclude the possibility that another region of the precursor molecule contains sugar residues whose conformation prevented binding to the columns. In contrast to the 14K preproglucagons, the 16K precursor was co-translationally processed and segregated to a slightly lower molecular weight proglucagon molecule, results which suggest the removal of an NH₂-terminal signal peptide^{8,11}. Thus, it would be of considerable interest to investigate the different modes of post-translational processing that the various processed and segregated proglucagon molecules undergo, particularly with respect to prohormone conversion to authentic glucagon.

During the preparation of this manuscript, a 14.5K precursor to angler fish proglucagon was reported²⁰.

We thank Dr R. Chance (Lilly) for gifts of bovine glucagon, pancreatic polypeptide and rabbit antiserum to bovine pancreatic polypeptide serum, and Gary Weiss for helpful discussions. This work was supported by NIH research grant AM-21860 to D.S. and a career development award from the Juvenile Diabetes Foundation. T.G.W. is a post-doctoral fellow of training grant AM-07329 and S.E.R. is a predoctoral fellow of the NSF.

Received 19 August; accepted 21 November 1980.

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Relationship of glicentin to proglucagon and glucagon in the porcine pancreas

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We have previously¹ isolated from porcine small intestine a peptide known as glicentin. The C-terminal portion of glicentin consists of the sequence of glucagon extended at its C terminus by an octapeptide^{2,3}, and differs slightly from the sequence of a proposed fragment of proglucagon⁴. Glicentin-like material has been demonstrated in the pancreatic A cell^{5,6}, wherein it is located in the periphery of the secretory granules⁷, whereas glucagon is located in the centre of the granules. To study the relationship of glicentin to the biosynthesis of glucagon, we have now investigated the glucagon-like and glicentin-like peptides in extracts⁸ and perfusates of the porcine pancreas. Our findings that a peptide with glicentin-like immunoreactivity, and intermediate in size between glicentin and glucagon, is secreted synchronously with glucagon suggest that this glicentin-related peptide is a major cleavage product of proglucagon.

Gel permeation chromatography of 10 extracts of porcine pancreas resolved the glicentin-like immunoreactivity into three peaks (Fig. 1): one (K_d 0.22 ± 0.01 ; mean $\pm$ s.e.m.) with an apparent size similar to that of glicentin; a second (K_d 0.44 ± 0.01), which accounted for the bulk of the immunoreactivity, intermediate in size between glicentin and glucagon; and a third (K_d 0.66 ± 0.01), which eluted in the leading edge of the glucagon peak. The first peak of glicentin-like immunoreactivity (K_d 0.22) was associated with glucagon-like immunoreactivity (GLI), implying that this peak could be similar to intact glicentin. The second (K_d 0.44) was not associated with GLI and could represent a fragment of glicentin which did not contain the sequence of glucagon. The third peak (K_d 0.66) could have possessed glicentin-like and glucagon-like immunoreactivities. The glicentin-related immunoreactivity and GLI recovered from these columns were immunometrically approximately equal.

Isoelectric focusing of an extract of porcine pancreas (Fig. 2) revealed one peak of glicentin-like immunoreactivity with a pI close to 4.0. This immunoreactivity was immunometrically equimolar with, but clearly dissociated from, the GLI, which had a pI of 6.4, the isoelectric point of porcine glucagon.

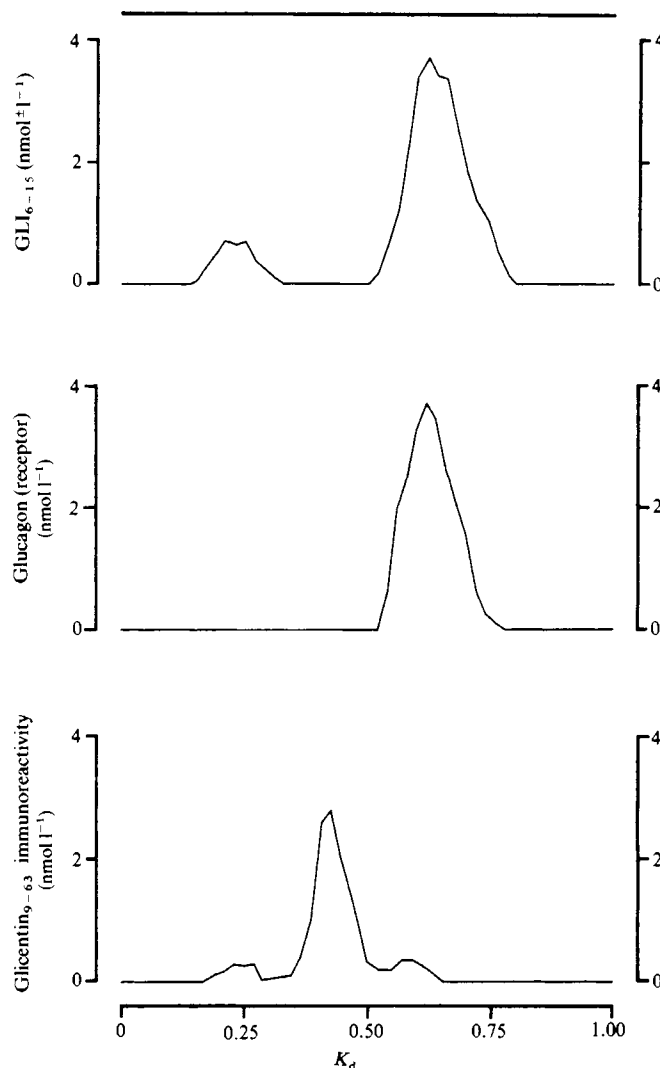


Fig. 1 Gel permeation chromatography of an extract of porcine pancreas on Sephadex G-50. Frozen porcine pancreas was extracted in acid-ethanol at -20°C , the extract centrifuged and 5 vol diethyl ether added. The peptide-containing aqueous phase was recovered and a sample corresponding to 190 mg of pancreas applied to a $1.6 \times 90\text{-cm}$ column of Sephadex G-50 fine equilibrated with 0.5 mol l^{-1} acetic acid. The radio receptor assay for glucagon was carried out using porcine liver cell membranes¹³. The glucagon-like immunoreactivities (GLIs) were assayed by the radioimmunoassay for glucagon according to Heding¹⁴. Anti-glucagon serum 4304 was used to measure GLI homologous with glucagon₆₋₁₅ (ref. 11). Anti-glicentin serum R64 (which reacts with the N-terminal portion of glicentin) was used in a radioimmunoassay for glicentin to measure glicentin-like immunoreactivity. The immunoassay consisted of incubating $100\text{-}\mu\text{l}$ aliquots of standards of porcine glicentin, or of unknowns, with equal volumes of antiserum diluted (1:65,000) in a 1:200 dilution of non-immune rabbit serum for 72 h. ^{125}I -glicentin ($100\text{ }\mu\text{l}$ of a solution containing $60\text{ pg }^{125}\text{I}$ -glicentin per ml) was then added and the incubation continued for 24 h. The free and antibody-bound ^{125}I -glicentin were separated by centrifugation 24 h after the addition of $100\text{ }\mu\text{l}$ anti-rabbit IgG serum (Dako). In these conditions, R64 does not react significantly with extracts of rat or human pancreas, or with vasoactive intestinal polypeptide, glucagon or gastric inhibitory polypeptide.

To examine further the relationship between porcine pancreatic glicentin-like immunoreactivity and glucagon, we have measured the secretion of these materials by the perfused porcine pancreas. If the glicentin-related peptides and glucagon are located within the same secretory granules—as indicated by the immunocytochemical findings—the secretory patterns of these two materials should be synchronous. Furthermore, if the glicentin-related peptides are cleavage products of glucagon biosynthesis, which are stored and then released together with

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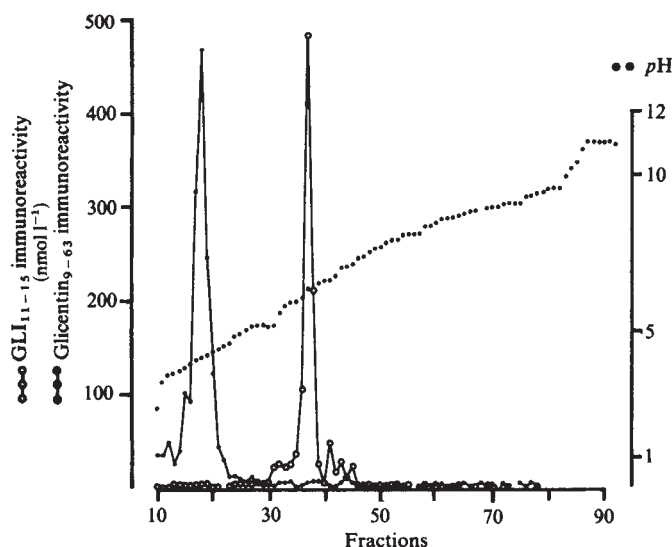


Fig. 2 Isoelectric focusing of an extract of porcine pancreas. A single porcine pancreas was carefully freed from adherent intestine, extracted in acid alcohol and the peptides recovered from the extract as described previously¹. The peptide pool was desalted on a column of Sephadex G-50 equilibrated with 150 mmol l⁻¹ acetic acid. The total peptide pool was recovered from the column and freeze-dried. The freeze-dried material, corresponding to 40 g wet weight of pancreas, was dissolved in ampholine/water and fractionated on a 440-ml LKG isoelectric focusing column with a pH 3.5–10 mixture of ampholines¹⁵ (LKB). The column was emptied in 5-ml fractions, and the glicentin-like immunoreactivity and GLI in the fractions were assayed as described in Fig. 1 legend, except that anti-glucagon serum K 4023 was used to measure GLI homologous with glucagon_{11–15}.

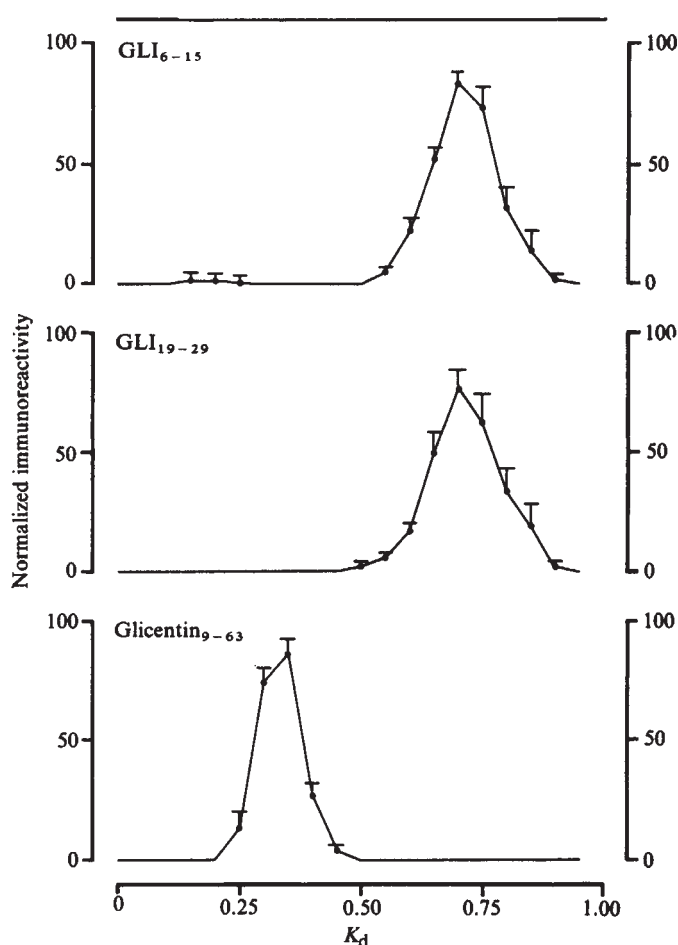
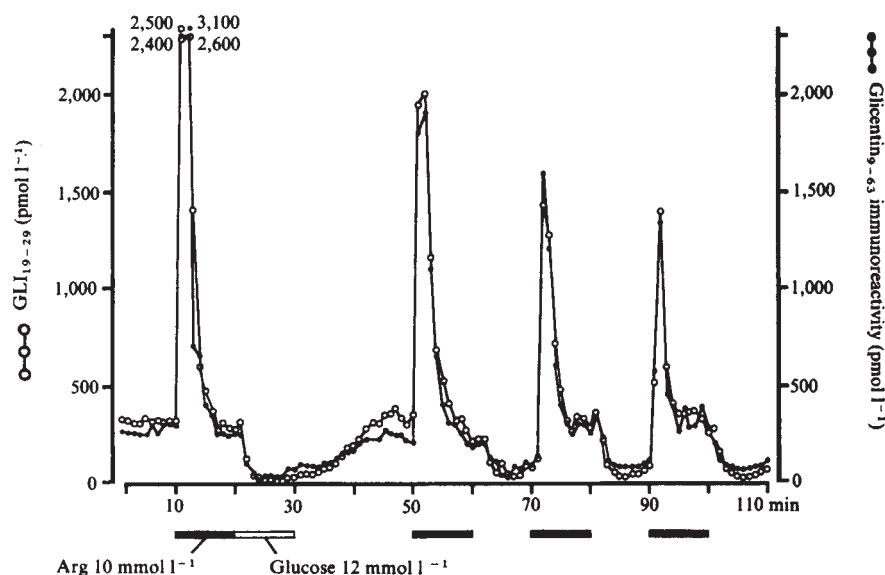


Fig. 4 Gel permeation chromatography of pancreas perfusates on Sephadex G-50 fine. Seven samples of perfusates taken from five different perfused pancreata stimulated with 5 mmol l⁻¹ arginine were applied separately to columns of Sephadex G-50 fine equilibrated with 150 mmol l⁻¹ NaHCO₃ containing 100 mmol NaCl, 20 µg ml⁻¹ human serum albumin and 600 µmol l⁻¹ thiomersal. The glicentin-like immunoreactivity and GLIs in the effluents were assayed as described previously. The data are the means (+s.e.m.) of the data from seven columns; the relative amounts of immunoreactivities recovered from each column were normalized as follows. The immunoreactivity recovered from each fraction (corresponding to a K_d interval of 0.05) was calculated for each column. The largest amount of immunoreactivity (irrespective of the assay used) recovered in any fraction from the column was taken to be 100, and the immunoreactivities in the other fractions for the column converted to a percentage of this value.

Fig. 3 The effects of arginine and glucose on the secretion of glicentin-related peptides and glucagon by the perfused isolated porcine pancreas. A porcine pancreas was isolated and perfused as described previously¹⁶. Glucagon release was stimulated by infusion of arginine into the arterial catheter to give a final concentration of 10 mmol l⁻¹ and suppressed by the infusion of glucose (12 mmol l⁻¹). GLI and glicentin-like immunoreactivity in the perfusate were assayed as previously described, except that anti-glucagon serum K 4305 was used to measure GLI homologous with glucagon_{19–29} (ref. 11).



glucagon, glicentin-related peptides and glucagon should be secreted in approximately equimolar amounts. Both these suppositions have been found to be correct: stimulation of the glucagon secretion of the perfused porcine pancreas with 10 mmol l^{-1} arginine caused synchronous and immunometrically equivalent increases in the secretion of glicentin-related immunoreactivity and of glucagon (Fig. 3). Gel permeation chromatography of perfusates from pancreases stimulated with arginine showed that the perfusates contained only one glicentin-related peptide, which was larger than glucagon (measured with anti-glucagon sera that reacted with the N- and C-terminal portions of glucagon) and which was devoid of GLI (Fig. 4). Isoelectric focusing of perfusate taken after a stimulation with arginine showed that the glicentin-related material had a pI of 4.0. The glicentin-related peptide secreted by the perfused pancreas was therefore similar to the major component of the glicentin-related peptides extracted from the pancreas.

The pancreatic glicentin-related peptides described here can be related to the biosynthesis of glucagon as follows. The peptide with the largest molecular weight, and which was found in small amounts in the pancreas, was very similar to glicentin and could be a stable early intermediate in the biosynthesis of glucagon, that is, proglucagon. The peptide which formed the bulk of the pancreatic glicentin-related material, and which was secreted synchronously with glucagon, could be a major fragment of proglucagon formed during the excision of glucagon from proglucagon. The existence of such a major fragment of proglucagon which is devoid of GLI has been described in rat islets⁹.

Final proof of this proposal requires further knowledge of the structure of pancreatic glicentin-related peptides, and a direct demonstration of their relationship to the biosynthesis of glucagon. Supporting evidence for this proposal, which suggests that the biosynthesis of gut GLIs in the intestine and of glucagon in the pancreas share common precursors, is provided by the findings that identical glucagon-containing peptides are found in the rabbit intestine and pancreas¹⁰, that glicentin can be converted into glucagon¹¹⁻¹⁷ and glucagon¹⁹⁻²⁹ by trypsin and carboxypeptidase B¹¹, and that ileal glicentin-containing 'L' cells develop glucagon C-terminal immunoreactivity after exposure to the same enzymes¹².

The findings that the pancreas stores and secretes a glicentin-related peptide, which is a possible fragment of proglucagon, suggest that the pancreatic A cell, like the B cell, stores and secretes a large fragment of the precursor molecule of its hormonal secretory product. Further investigation of the nature of pancreatic glicentin-related peptide is required to establish the biological significance of its co-secretion with glucagon.

We thank Karin D. Jørgensen for providing fresh porcine pancreas, and K. Christensen, D. Petersen, W. Jørgensen and E. Gammelgaard for technical assistance.

Received 10 September; accepted 21 November 1980.

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Molecular cloning and organization of two leghaemoglobin genomic sequences of soybean

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The leghaemoglobins (Lb) are myoglobin-like proteins found in all nitrogen-fixing root nodules of legumes¹⁻³. They are encoded by plant nuclear genes⁴ which are specifically induced and form the predominant protein in nodules developed in symbiosis with the appropriate species of *Rhizobium*. The Lb is located in the host-cell cytoplasm of the infected cell⁵ and is thought to facilitate oxygen diffusion^{6,7}. Amino acid sequencing of the soybean Lbs has revealed at least four primary structures differing only in a few amino acids⁸⁻¹⁰. We have previously estimated about 40 copies of Lb sequences in the soybean (*Glycine max* L.) genome by cDNA hybridization⁴. To investigate Lb gene organization and function, we prepared and characterized a Lb cDNA recombinant molecule, pLb1, and used it to isolate two genomic Lb sequences from a library constructed in Charon 4. We report here that the organization of the two genomic Lb sequences is quite distinct and one of them seems to have an intervening sequence(s). Hybridization of pLb1 with genomic DNA from various tissues showed that Lb sequences are dispersed through more than 30 kilobases of genomic DNA and that there is no apparent sequence rearrangement or methylation changes following induction of Lb genes.

Double-stranded cDNA prepared to nodule RNA was digested with *Sau*3A restriction endonuclease and ligated into the *Bam*HI site of the plasmid pBR322 (see Fig. 1 legend). Clones containing Lb sequences were identified by their ability to hybridize with soybean root nodule cDNA, by their hybridization to 9S RNA from root nodules and lack of hybridization to RNA from roots, and by their specific hybridization to sequences in root nodule RNA which are translated into Lb protein in a wheat-germ protein synthesis system (data not shown).

Restriction enzyme analysis of one of the Lb clones demonstrated that the insert was about 145 base pairs long, contained a site for *Alu*I (but not for *Hae*III, *Taq*I or *Hin*I) and was bounded by sites for *Bam*HI. (The appropriate bases required to regenerate the *Bam*HI site at each end of the insert must have been adjacent to the *Sau*3A sites in the cDNA.) The *Alu*I site corresponds to the position of an *Alu*I site found in a previously described Lb cDNA clone¹¹. The DNA sequence of the insert and the predicted amino acid sequence are shown in Fig. 1, together with a partial amino acid sequence of the soybean Lb_{c2}, c₁ and Lb_a. The sequence of the cloned DNA encodes from amino acid residue 99 to the carboxy-terminal phenylalanine (residue 143) of Lb_{c2} with the exception of an additional valine between residues 102 and 103, and the substitutions of Asp for Asn and Gln for Glu (positions 99 and 101 of the Lb_{c2} sequence⁸, respectively). The amino acid sequences of Lb_{c1} (Fig. 1e) and Lb_a (Fig. 1f) differ in 4-8 amino acid residues at this end of the molecule. Thus the cloned Lb sequence represents either a variant of Lb_{c2}, for example, Lb_{c3} (see ref. 12) or the minor differences may have arisen during molecular cloning¹³.

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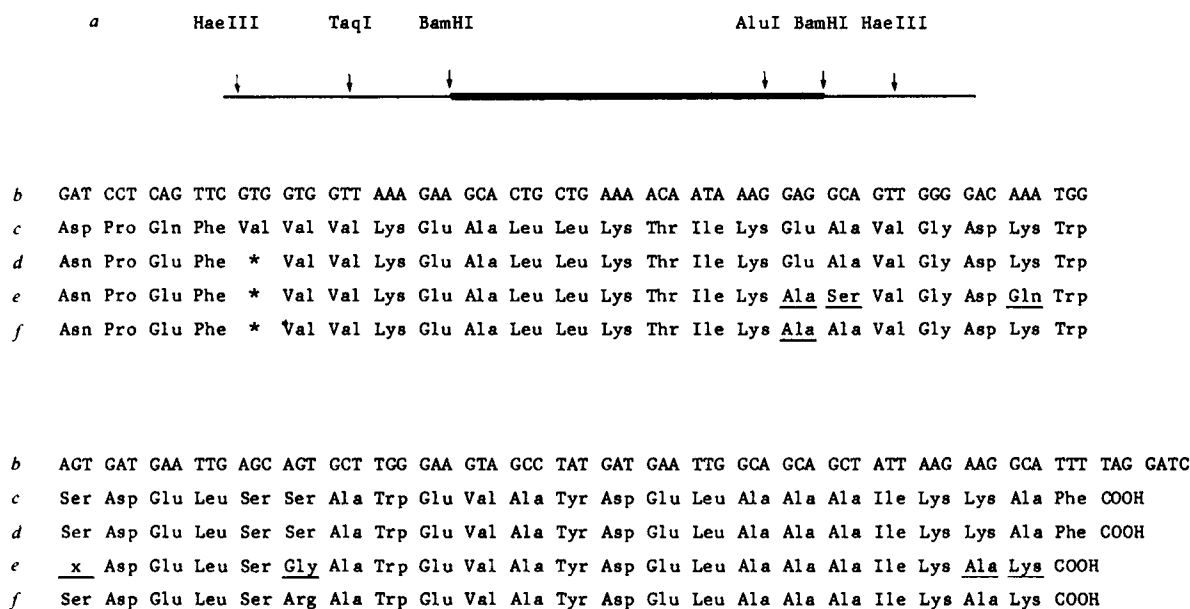


Fig. 1 Characterization of the double-stranded cDNA clone, pLb1. **a**, Diagram of pLb1 insert area; heavy line is cDNA sequence, light lines are pBR322 sequences. **b**, Nucleotide sequence of the 145-base pair insert. **c**, Predicted amino acid sequence encoded by **b**. **d**, **e** and **f**, amino acid sequences of soybean Lb c₂, c₁ and a, respectively⁸⁻¹⁰. The amino acids underlined in **e** and **f** are different from those in **d**. cDNA and double-stranded cDNA¹⁶ were synthesized with reverse transcriptase from oligo (dT)-bound RNA isolated from 3-week-old soybean root nodules¹⁷ (*Glycine max* var. Prize infected with *Rhizobium japonicum* strain 61A76). *Sau3A*-cleaved double-stranded cDNA (10–50 ng) was ligated to *Bam*HI-cleaved pBR322 (1 µg) in 5 µl of 20 mM Tris HCl (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM ATP and 0.1 U T₄ DNA ligase (BRL) at 10 °C for 12 h. *Escherichia coli* strain 490 or HB 101 cells were transformed¹⁸ and ampicillin (25 µg ml⁻¹)-resistant and tetracycline (20 µg ml⁻¹)-sensitive colonies were selected by replica plating on media containing the appropriate antibiotic. Colonies were screened for leghaemoglobin sequences by hybridizing replica filters¹⁹ with ³²P-labelled cDNA in 0.6 M NaCl, 50 mM HEPES (pH 7.8), 100 µg ml⁻¹ heat-denatured sonicated salmon sperm DNA, 0.5% SDS and 50% formamide at 37 °C. Further characterization of positive clones is discussed in the text. The pLb1 insert, including pBR322 sequences, bounded by *Hae*III sites, was isolated by polyacrylamide gel electrophoresis, cut with *Taq*I, and sequenced by the methods of Maxam and Gilbert²⁰.

Hybridization of restriction enzyme-digested genomic DNA with nick-translated pLb1 reveals several bands (Fig. 2a) consistent with the idea of a family of genes⁴. The hybridizing sequences of pLb1 are found within at least 10 regions of the soybean genome differing in their *Eco*RI sites (Fig. 2a, tracks 1 and 2). A similar diversity of bands is seen in *Hha*I- (Fig. 2a, tracks 3 and 4) and *Hind*III-cleaved (Fig. 2a, tracks 5 and 6) genomic DNA. The total amount of DNA contained in these fragments ranges from about 30 kilobases (*Hha*I fragments) to almost 50 kilobases (*Eco*RI fragments). The more intensely hybridizing bands (for example, the 1.4- and 4.2-kilobase bands in tracks 1 and 2) may be explained by multiple pLb1 sequences within these fragments or by multiple genes containing the same fragment size. Thus the family of Lb genes is dispersed in the soybean genome and is not composed primarily of identical tandem repeats.

The fragment patterns and intensity of the bands are basically identical whether the DNA was isolated from embryos or nodule tissue. This suggests that the Lb genes are not specifically amplified or rearranged in nodule tissue developed as a result of infection by *Rhizobium*⁴. Furthermore, the *Hha*I results (in addition to those of *Msp*I and *Hpa*II, data not shown) indicate that the induction of the Lb genes is not accompanied by major changes in methylation at these sites.

A soybean genomic library constructed in Charon 4 was screened with cDNA and pLb1 and yielded two clones which contain hybridizing *Eco*RI fragments of ~11.5 (Ch4 Gm Lb11) and 4.2 (Ch4 Gm Lb4) kilobases (Fig. 2b, lanes 2 and 3). These fragments correspond to the 11.5 and 4.2-kilobase bands observed in *Eco*RI-cleaved genomic DNA. Both clones contain approximately 14 kilobases of genomic DNA but those sequences which hybridize to the Lb probes are within quite distinct restriction-endonuclease domains (Fig. 3). The use of Ch4 Gm Lb11 and Ch4 Gm Lb4 as hybridization probes to *Eco*RI-cleaved genomic DNA reveals bands which correspond to the *Eco*RI bands in the respective Charon clones, as well as two to four additional bands (data not shown). The relative

intensities of the bands indicate approximately equimolar amounts of the fragments in the genome and, when compared with the intensity of hybridization of a ribosomal DNA recombinant probe¹⁴, each band seems to be present in fewer than 10 copies per haploid genome.

The general organizational maps of the two clones were constructed by various single and multiple restriction-enzyme digestions followed by blotting and hybridization (Fig. 3a, b). A more detailed map of Ch4 Gm Lb11 was obtained by sequential hybridization with pLb1 followed by cDNA to reveal both 3' and 5' ends of the sequences. pLb1 sequences represent the 3' end of the coding region of Lb mRNA (Fig. 1); a complete cDNA probe would represent sequences predominantly to the 5' end of pLb1. Representative data are given in Fig. 2c. Tracks 1 and 6 (*Msp*I and *Hae*III) clearly show the presence of two sequences which are hybridized by both pLb1 and cDNA (1.3 and 1.7 kilobases, track 1) and a 1.6-kilobase band hybridized exclusively to cDNA (track 6). Tracks 2–5 and 7–10 show similar data on pLb1 and/or cDNA hybridizations for the enzyme combination *Mbo*II plus *Hae*III. The fragment hybridized exclusively by cDNA is reduced from about 400 base pairs to 300 base pairs. Applying this sequential hybridization strategy with several restriction enzymes has allowed us to construct the detailed map of Ch4 Gm Lb11 shown in Fig. 3c. This map is confirmed by the Smith and Birnstiel²⁶ method of 5'-end labelling of DNA. Note that the separation of the sequences which hybridize exclusively to cDNA from the nearest pLb1 hybridizing sequences is ~1.3 kilobases. Another region containing pLb1 hybridizing sequences is at least 1 kilobase further away. The region hybridized by the cDNA spans up to 5 kilobases. If the sequences in Ch4 Gm Lb11 represent a complete or even a partial gene, there must be at least one intervening sequence within the Lb coding region. Sufficient DNA to encode the entire mRNA is contained within the exclusively cDNA hybridizing and the nearest pLb1 hybridizing regions. The present data do not allow us to limit the positions of cDNA and pLb1 hybridization sequences, particularly those to the right of the

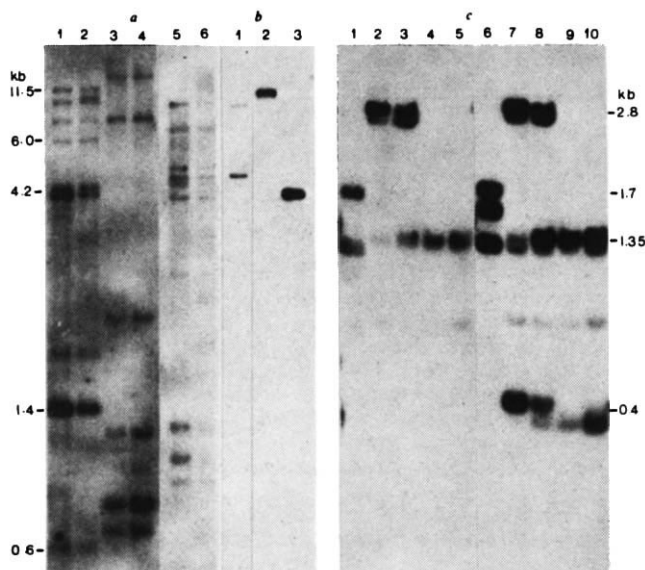


Fig. 2 Analysis of total genomic and cloned genomic Lb sequences. *a*, Restriction endonuclease-digested soybean DNA (5 µg per track) or *b*, *c*, recombinant clone DNA (~0.5 µg per track) was separated by agarose gel electrophoresis, blotted on to nitrocellulose paper²¹ and hybridized with ³²P nick-translated²² pLb1 at ~10⁸ c.p.m. µg⁻¹ and root nodule cDNA (*c*, tracks 6–10) at ~10⁷ c.p.m. µg⁻¹. Hybridization conditions were 6×SCP (1×SCP = 0.1 M NaCl, 0.03 M Na₂HPO₄, 0.001 M Na₂ EDTA, pH 6.2), 1% Sarkosyl, 2.5% Dextran sulphate at 68 °C for 24–48 h. Exposure of the hybridized filter to pre-fogged X-ray film at –70 °C with intensifying screens was for *a*, 6 d, or *b*, *c*, 12–24 h. Fragment sizes (in kilobases (kb)) were determined from the positions of *Eco*RI-cleaved λ phage and *Taq*I-cleaved pBR322. *a*, Soybean DNA extracted from embryos (track 1) or nodule tissue (track 2) was digested with *Eco*RI; tracks 3 and 4 were digested with *Hha*I; tracks 5 and 6 with *Hind*III. *b*, Ch4 Gm Lb11 DNA digested with *Hind*III (track 1) or *Eco*RI (track 2); Ch4 Gm Lb4 DNA digested with *Eco*RI (track 3). *c*, Ch4 Gm Lb11 DNA digested with *Hae*III and *Msp*I (tracks 1 and 6) or *Mbo*II followed by *Hae*III partial digestions (tracks 2–4 and 7–9) and complete *Hae*III digestion (tracks 5 and 10). Tracks 1–5 were hybridized to pLb1 and 6–10 to pLb1 followed by Lb cDNA. The genomic recombinant molecules containing Lb sequences were isolated from a library constructed by R. Fisher and R. Goldberg in Charon 4 with partial *Eco*RI-digested soybean DNA by the procedures of Maniatis *et al.*²³. About 400,000 plaques were screened²⁴ with ³²P-labelled cDNA and pLb1 and three 'positives' were plaque purified²⁵. Two of these had identical *Eco*RI and *Hind*III sites and have been designated Ch4 Gm Lb11 in reference to the size of the *Eco*RI fragment which is hybridized to pLb1. The third recombinant (Ch4 Gm Lb4) contains a 4.2-kilobase *Eco*RI fragment that hybridized to pLb1.

*Msp*I site. With the above organization of Lb gene, the direction of transcription would be from left to right. In comparison with Ch4 Gm Lb11, the Lb4 has a very restricted region of hybridization with both cDNA and pLb1 indicating that it may not have an intervening sequence or it contains a partial gene sequence. The Lb proteins are very similar^{8–10} (see Fig. 1*d, e, f*) and the cDNA and mRNA sequences seem to cross-hybridize fully⁴. Thus, the genomic sequences hybridized to pLb1 are expected to include all the Lb genes, and Lb clones cannot be identified as encoding any specific Lb protein without sequencing the DNA. Finer mapping and sequencing of fragments could also demonstrate the interrelationship between these genes as well as among other isolated Lb genes in the family.

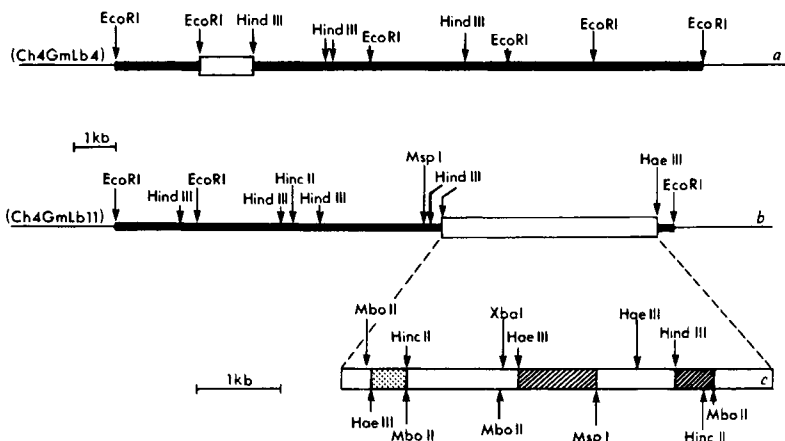
The organization and regulation of the Lb genes is of interest at several levels¹⁵: (1) the basic molecular biology of a higher plant nuclear gene; (2) the elegantly orchestrated interactions between the plant and the prokaryote which induces these genes, and (3) the significant agricultural importance of the legume/*Rhizobium* association leading to the fixation of atmospheric nitrogen.

We thank Dr D. Housman for helping to construct and manipulate the Charon libraries and Dr R. Goldberg for providing a soybean library. This study was supported by grants from the Rockefeller Foundation and the Natural Sciences and Engineering Research Council of Canada. The recombinant DNA work was carried out in a B-level facility in accordance with the guidelines of the MRC Canada.

Received 8 August; accepted 19 November 1980.

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Fig. 3 Organizational map of two Lb genomic sequences. *a, b* represent entire insert (heavy line) containing regions of pLb1 hybridization (open box) representing 1.3 and 5 kilobases (kb) in Ch4 Gm Lb4 and Ch4 Gm Lb11, respectively. Light lines represent the arms of Charon 4. *c*, Detailed map of Ch4 Gm Lb11 containing Lb sequences. The dotted area represents sequences exclusively hybridized to Lb-cDNA whereas hatched areas contain sequences hybridized to pLb1.



BOOK REVIEWS

Fast breeders: all cons, no pros?

Alvin M. Weinberg

THAT nuclear energy would grind to a halt unless a breeder were developed was understood by the original workers in nuclear energy. What was not clear then, and what is still not entirely clear, is the kind of breeder that ought to be developed. In 1952, Bennett Lewis, father of the Canadian CANDU system, along with E. O. Lawrence, proposed the electric breeder based on an enormous proton accelerator. A variant of electric breeding, the so-called fission/fusion hybrid is now being discussed fairly seriously. I, along with H. G. MacPherson, espoused breeders based on the thorium cycle, particularly those using molten salts. And a thorium breeder based on light-water reactor technology has been built by H. G. Rickover in Shippingport, Pennsylvania. But the plutonium fast breeder, pioneered by W. H. Zinn, gained momentum and is now essentially the only show in town.

We could always give a plausible rationale for the ultimate deployment of breeders, but we could never say when breeders, of whatever kind, would be necessary. Since the fuel cycle cost of the breeder is far less sensitive to the cost of the raw uranium than is the fuel cycle cost of non-breeder pressurized water or gas-cooled reactors, eventually breeders would provide cheaper energy than would non-breeders. Our argument for aggressive development and quick deployment of the breeder was therefore based on the hope that breeders might be cheaper than non-breeders. The only way to find whether or not this was the case was to build large breeders. This remains the strongest argument for vigorous development of a full-size fast breeder. We had two other, albeit secondary, arguments favouring quick deployment of breeders: they would confer on their users a measure of energy autarky and thus would relieve pressure on non-renewable energy sources; and their deployment would put a virtual end to the mining of uranium, the only part of the fuel cycle that poses a technically difficult problem of waste disposal.

The basic economic arguments have hardly changed in the intervening 30 years, as these proceedings of the Polytechnic of the South Bank 1978 Conference on the Fast Breeder demonstrate. The main change is the emergence of non-economic

The Fast Breeder Reactor: Need? Cost? Risk? Edited by Colin Sweet. Pp.232. (Macmillan Press: London, 1980.) £20.

issues — political, social and ethical — that were barely identifiable in the early days. As the editor, Colin Sweet, says,

It is necessary to subject the official view to a critical assessment and it was the purpose of the conference . . . to do that . . . the issues . . . go far beyond what is to be found in the official literature . . . There is no question of trying . . . to strike 'a balanced view,' because there is no way in which such a phrase can be interpreted.

The resulting 14 papers and five appendices examine not only the old questions (will the fast breeder be economic; and will it be safe?) but also the far broader question of whether nuclear energy itself is needed. Most of the authors are opposed to nuclear energy. Their attack on the fast breeder is an attack on nuclear energy itself. When David Widdicombe argues that nuclear energy and civil liberties are incompatible or when Professor Patricia Lindop says that "the radiation hazard to the population may prove to be the factor limiting the utilization of nuclear energy" they are calling for an end to nuclear energy, not merely an end to the fast breeder.

For those seeking a balanced account of the debate over the fast breeder reactor, these proceedings will be disappointing: it is not only that the positions are far too polarized, it is that there is no real confrontation between the different points of view. Though the nuclear advocates are called to task, their opponents always have the last word. I find those last words, such as Peter Odell's claim of 70% efficiency for oil burning heaters, incomplete and tendentious; they scarcely conceal a profound distrust of nuclear energy.

The editor of *Nature*, in asking me to review this book, implied that the fast breeder reactor issue in the United Kingdom is controversial and thus that the book would be best dealt with by an outsider. As an American, I am reluctant to become involved in a family squabble. Nevertheless, for what it is worth, I offer these points for consideration.

(1) The denial of nuclear energy amounts to a denial of human ingenuity.

For example, much is made of the loss of civil liberties entailed in nuclear energy. Yet, since Strategic Air Command bases can be made secure with minimal infringement on civil liberties, I don't see why the same cannot be done for nuclear sites.

(2) I have never been a strong supporter of the fast breeder reactor; I have always thought the thermal system based on thorium was a better bet. In the best of all possible worlds, many different breeders would be pursued, and the choice of the fast breeder would not have pre-empted development of the alternatives to the point where a fair comparison could be made between the various possibilities.

But in the real world, the fast breeder is now practically the only option. Despite the real possibility that fast breeders will prove too costly to make economic sense in the short run, active development of the breeder is at least as sensible as is active development of solar energy. The two are the only inexhaustible energy sources that we know to be technically feasible. We shall know the price of the breeder only by building several, just as we shall know the price of solar power towers only after we have built some.

(3) In the final analysis, we must decide whether the risk in going ahead with the fast breeder and then discovering we do not need it (fusion may work, or solar may be cheap) is less than the risk of not going ahead with the breeder and discovering in, say 2025, that we need it. Considering what is at stake — our energy future, not to speak of the spectre of CO₂-induced climate change — I believe we must go ahead with development of the breeder, expensive though this may be.

France, which has chosen to generate 40 per cent of its primary energy from non-fossil sources by 1990, has decided to depend on nuclear energy — that is, on the breeder. It is not unlikely that, in the year 2000, the rest of us may envy the degree of energy autarky enjoyed by France as a result of this decision while we bicker over whether nuclear energy can be mended, and fight over the oil that remains in the Middle East. □

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Conservation: the hopes and the fears

Brian Bertram

Conservation Biology: An Evolutionary-Ecological Perspective. Edited by Michael E. Soulé and Bruce A. Wilcox. Pp.395. (Addison-Wesley/Sinauer: 1980.) Pbk £7.95, \$14.50.

THIS will be a disturbing book to anyone who believes that conservation is a matter merely of defining and protecting a number of reserve areas which are representative of ecosystems being destroyed. Michael Soulé and Bruce Wilcox have gathered a fine series of papers demonstrating the vast problems ahead of us all, and showing what we can expect the results of our conservation efforts to be.

Part I contains four chapters on the ecological principles of conservation. They concentrate on tropical forests, which are particularly vulnerable and of which it is likely that two-thirds will have disappeared within a mere 20 years. These early contributions by Gilbert, Eisenberg, Diamond and Foster, show what it is that we risk losing — a fabulously intricate set of interactions among plants and animals, varying both with locality and over time. We are clearly shown that small isolated "representative" portions of the vanishing tropical forests do not contain or cannot maintain more than a fraction of the species of the whole area represented.

Isolated reserves dotted about in a barren waste of altered or destroyed habitat act like islands; the five chapters in Part II consider the consequences of this "insularization" (horrid word). It is well known that true islands generally contain fewer species than the adjacent mainland, and a frequent cause is that the small populations on small islands are highly vulnerable to extinction. Recolonization depends on accessibility from other land areas. Wilcox, and Terborgh and Winter, draw on principles from insular ecology and extrapolate to isolated reserves.

Even if they do not become extinct, small populations face unusual evolutionary problems, as is shown in Franklin's and Soulé's chapters. They are subject to genetic drift, and they contain only a limited amount of genetic variability with which to respond to selection pressures. The level of inbreeding among members of the small population rises, fixing deleterious recessive genes and resulting in higher mortality, lower fecundity and distorting the sex ratio towards surplus males. Two particularly important conclusions emerge. First, that the effective population size must be not less than about 50 individuals to keep inbreeding to a level low enough for the population to survive. Second, that in Soulé's words "significant evolutionary change in most higher organisms is coming to a screeching halt in the tropics".

Part III, on captive propagation and conservation, deals with the similarly small populations which can be kept and bred in zoos. Some species can certainly be preserved in captivity even when extinct in the wild. But, as Conway shows, all the zoos in the USA together contain only enough space for viable populations of about 200 species of mammals. Coordination among zoos is required and is growing, but recognition by society of their important role is far too slow. Benirschke and colleagues describe some of the variety of modern aids in captive propagation, and Kleiman surveys the application of socio-biological study to animals in captivity; both enable greater breeding potential to be realized. Successful reintroduction to the wild is the ultimate and difficult objective, and Campbell reviews the numerous problems involved and the successes achieved.

The final section in the book deals with exploitation and strategies of conservation. Coe examines ecological interactions between human beings and wildlife in Africa, and assesses the present and future importance of game hunting, pastoralism, the tsetse fly, tourism, and the prospects for the culling, ranching and domestication of wild species of mammal. With Kenya's population now increasing at 4% per year, major problems are looming fast. Whitmore describes the extent and consequences of the destructive exploitation of tropical forests — some 50 hectares have been destroyed and disappeared while you have been reading this review, and another 30 will have gone before you finish. The direct effect on local species of animal is obvious; the indirect effects on all animals everywhere, because of the climatic changes caused, are unknown.

What we should do is the subject of the last two chapters. Pyle discusses the general management of nature reserves in the developed world. But that is not where most of the problem lies, although many of the causes of the problem do, according to Ehrlich. Human population growth threatens all tropical habitats; but so too does economic growth or the expectation of it. The Amazon forests are being erased only partly to provide living land for burgeoning Brazilians but also to provide timber and beef for North America and Europe. Ehrlich urges us to initiate and follow a more aggressive and wider conservation strategy. We must not be misled by the relatively slow rate of habitat loss in our own countries.

The papers in this book are diverse in character, but they all point very clearly in one direction. The editors summarize it eloquently:

The green mantle of Earth is now being ravaged and pillaged in a frenzy of

exploitation by a mushrooming mass of humans and bulldozers. Never in the 500 million years of terrestrial evolution has this mantle we call the biosphere been under such a savage attack. . . There is no escaping the conclusion that in our lifetimes, this planet will see a suspension, if not an end, to many ecological and evolutionary processes which have been uninterrupted since the beginnings of palaeontological time. We hope it is only a suspension — that the horrible onslaught can be stopped before the regenerative powers of ecosystems are killed. This book is a statement of that optimism. . . This is the challenge of the millenium. For centuries to come, our descendants will damn us or eulogize us, depending on our integrity and the integrity of the green mantle they inherit.

This book is not the uninformed emotional outburst of an eco-freak. It is a collection of the informed statements and pleas of 21 experts. But it is well written, so anyone can read it. And everyone should. □

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Weather and water

William R. Cotton
Kevin R. Knupp

Clouds and Storms: The Behaviour and Effect of Water in the Atmosphere. By F.H. Ludlam. Pp.405 + plates. (Pennsylvania State University Press: 1980.) £24.95, \$57.50.

THE late Dr Frank H. Ludlam was a keen observer of nature. His ability to combine and synthesize fragments of data and thereby construct data-consistent conceptual models of weather-phenomena is unmatched in this century. In this book, Dr Ludlam focused on the various forms of water in the atmosphere as the underlying factor in controlling the unique characteristics of many weather systems.

The book is largely devoted to the observed characteristics of the physics and dynamics of convective clouds (Chapters 5, 7 and 8). Discussion of topics closely related to cloud processes — radiation, atmospheric optics, atmospheric thermodynamics, atmospheric aerosol physics and cloud microphysics — in the preceding chapters serves as a useful background. A transition chapter (6) compares the time-scale and energy of clouds to related atmospheric processes. The ninth and final chapter deals with clouds associated with larger-scale systems.

The book is unique in several respects. First, it contains a copious amount of

information, particularly on observed details of convective clouds in Chapters 7 and 8. These two chapters alone contain 397 references to various world-wide studies, and provide an excellent reference list for those new and even well-established in the field. A second notable feature is the description of observed phenomena presented in the appendices (a total of 33) at the end of many of the chapters. Finally, the book is well illustrated with many figures and 88 photographs which depict the various physical phenomena described in the text.

It is unlikely that this book will serve as the main textbook for a course in cloud physics, cloud dynamics or mesoscale aspects of clouds or cloud systems. Cost alone would prohibit its general use as a class textbook. Without prior training in dynamic meteorology, radiative transfer theory and cloud physics, an undergraduate would find many of the discussions hard to follow and would be overwhelmed by the sheer volume of the material. As a graduate-level text,

however, the book is limited by the lack of theoretical depth. Much of the theory in cloud physics, radiative transfer theory and cloud dynamics is that which was current during the late 1950s and mid-1960s.

To the lecturer, the book will provide excellent, easily accessible material for preparing lectures in cloud physics and cloud dynamics. Ludlam's descriptions, together with the appropriate illustrations, give form to physical concepts and provide insight into the processes governing the behaviour of clouds and related phenomena in the atmosphere.

Because this book embraces a variety of observational information not available in other texts on cloud physics or atmospheric dynamics, it will be a valuable resource book for the serious student, researcher or teacher of cloud physics or cloud dynamics. □

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Learning from nematodes

J. Hodgkin

Nematodes as Biological Models. Edited by Bert M. Zuckerman. Vol.1, *Behavioral and Developmental Models*, pp.321; Vol. 2, *Aging and Other Model Systems*, pp.306. (Academic: 1980.) Each volume \$35, £19.60.

IN STUDYING any biological phenomenon, it is desirable to work on an organism which is simple, well characterized and amenable to experimental manipulation. For many purposes, nematodes satisfy these criteria, and consequently they have attracted much attention as model systems in which to examine a variety of problems in cell and developmental biology, neurobiology and ageing. One nematode species in particular, *Caenorhabditis elegans*, has been characterized in great detail over the past 15 years, and an enormous amount is now known about this animal. However, this information is scattered widely throughout the literature, or else remains unpublished, so a monograph reviewing research on *C. elegans* and related nematodes would be very valuable. These books only partly satisfy this need, although most of the first volume, and parts of the second, are devoted to work on the genus *Caenorhabditis*. Each of the 18

Spectroscopy for the neophyte

H.A.O. Hill

An Introduction to Spectroscopy for Biochemists. Edited by S.B. Brown. Pp.403. (Academic: 1980.) £18.40, \$44.50.

THE avowed intent of the editor is to provide, in a single volume, a description of spectroscopic techniques at an advanced level appropriate to the non-specialist biochemist or clinician. How closely do the individual contributors approach this estimable, if unrealistic, goal in their presentations of "non-mathematical yet rigorous" accounts of particular techniques?

The "Introduction to Spectroscopy" by the editor provides the nervous clinician with a gentle, and certainly non-mathematical, survey of the major techniques, though the physical basis of the methods could be rather better explained. The following description of ultraviolet and visible spectroscopy by Dr Brown is clear and lucid, though I would have preferred to have selection rules discussed in terms of the probability of transitions taking place. Most spectroscopic techniques are best taught by illustrating their applications, and the spectra of amino-acids, nucleosides, nucleic acids, carotenoids, flavinoids and tetrapyrroles are briefly, but adequately, presented. By far the best part of the chapter is that concerned with applications to the analysis of biological materials.

G.R. Penzer provides a near-perfect pedagogical exposition of the principles of fluorescence — the only flaw being the in-

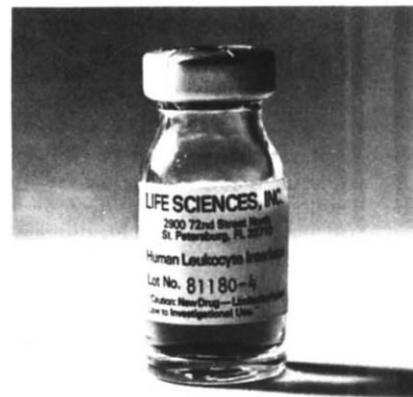
adequate number of examples — and the chapter on vibrational spectroscopy by P. Gans is well-balanced with most emphasis being placed, quite correctly, on the application of Raman, especially resonance Raman, spectroscopy. P. Bayley's account of circular dichroism and optical rotation is both rigorous and non-mathematical, is well illustrated and uses many well-chosen examples, though twice or thrice as many would not have been excessive.

R. Jones continues with a description of magnetic resonance spectroscopies. These are the most difficult techniques to explain, especially the EPR spectroscopy of metalloproteins. The temptation is to adopt the simplest description even when it is wrong. Thus, though crystal field theory as an *explanation* of the consequences of introducing metal ions into a non-spherical ligand environment died 20 years ago (some would say it was stillborn), it still infiltrates otherwise respectable essays.

The book is rounded off with chapters by E.J. Wood on atomic absorption spectroscopy and by A.J. Geddes on mass spectrometry which should be valuable to those intending to apply these methods for the first time. In short, this is a book that seeks to attain an impossible goal yet with much that is good in it. □

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chapters has a different author or authors, and there appears to have been little overall editorial control, so the contributions vary considerably in quality, purpose and organization. Few of them convey anything of the excitement, novelty or potential of research in this area.

The particular advantages of *C. elegans* are its neuroanatomy, development and genetics. The review of genetic analysis is excellent, and there are also adequate chapters on developmental genetics, biochemical genetics and the molecular genetics of muscle. The section dealing with cell lineages and development is long and thorough, but needlessly turgid. Neuroanatomy is scarcely described at all (apart from a good general chapter on nematode sense organs), although the total anatomy and connectivity of most parts of the nervous system have been known for some time. This omission is especially unfortunate in view of the chapter on behaviour, and the admirable summary of

work on *Ascaris* neurobiology. Thus there is still no opportunity of comparing the two species without going back to the original papers.

The second volume contains contributions on ageing and longevity, nutrition, respiration, osmoregulation, energy metabolism, and the structure of nematode cuticle and sense organs. Some of these chapters are accounts of nematological research, rather than the use of nematodes to investigate fundamental biological problems, and therefore seem inappropriate to these volumes. Nevertheless, there is a great deal of interesting material in the two books; they are up-to-date and Academic Press has done a good job on the reproduction of photographs. For the time being, these volumes provide a useful introduction to contemporary research using nematodes. □

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Genetics and plant populations

Donald A. Levin

Demography and Evolution in Plant Populations. Edited by Otto T. Solbrig. Pp.222. (Blackwell Scientific/University of California Press: 1980.) £15, \$29.50.

DURING the past decade, the discipline of plant demography crystallized from many disparate elements of empirical and theoretical ecology, and reproductive biology scattered in the basic and agricultural sciences. This discipline focuses principally on the quantitative aspects of germination, growth, reproduction, dispersal and death in a population, and the implications of these processes on the dynamics of population numbers and on life-history strategies. Although it is apparent that many characteristics of individuals related to the demography of populations are under genetic control, and that the demographic properties of populations may manifestly influence the genetic structure and amount and mobilization of genetic variation, the genetic perspective remains somewhat alien to scientists interested in plant demography. *Demography and Evolution in Plant Populations* is a step toward a unification of the ecological and evolutionary genetic components of demography.

The primary objective of the editor, Otto Solbrig, in assembling this collection of eight papers was to achieve a greater understanding of the dynamics of populations in relation to the action of natural selection. Papers by Abrahamson, Cook, Sarukhan and White deal almost exclusively with the dynamics of numbers of growth. One by Solbrig and another by

Lloyd are principally genetic in content. Only the paper by Snaydon broadly integrates genetic and numerical aspects of demography. The result is a volume about demography and about evolution, rather than about the reciprocal influences of numbers and genotypes in populations.

Although the volume does not provide a broad synthesis, it does contain informative treatments of some major topics in plant demography. The high points of the volume are the treatments of numbers and size in plants by White, and the genetic variable in demography by Snaydon. Both papers bring to light much agricultural information which ecologists and evolutionists are unlikely to encounter elsewhere. Sarukhan's contribution on demography in the tropics is also packed with new information and insight. In the other chapters, the biology of seeds in the soil is imaginatively reviewed by Cook; vegetative reproduction and the genet-ramet dichotomy are treated by Abrahamson; Lloyd discusses conditions favourable to self-fertilization in predominantly outbreeding species; and Solbrig reviews the genetic structure of populations, and highlights approaches which integrate demography and genetics.

Demography and Evolution in Plant Populations will be a useful and authoritative source of data and ideas about plant demography. Scientists interested in plant population dynamics, reproduction and growth will find the volume to be of considerable interest and value. □

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Tanford number two

B.A. Pethica

The Hydrophobic Effect: Formation of Micelles and Biological Membranes. 2nd Edn. By Charles Tanford. Pp.233. (Wiley: 1980.) £10.50, \$24.65.

THIS second edition of Dr Tanford's well-known book has been extensively rewritten to include recent advances in micelle studies, and contains a number of new chapters, most notably on serum lipoproteins and membrane proteins. It is not a review of recent results, but rather a synthesis or overview written in a lucid and occasionally magisterial style by a master of the subject.

The emphasis is on the phenomena of the hydrophobic effect, using thermodynamic arguments in a clear fashion which avoids pointless rigour. The accounts of light scattering, spectroscopic and similar methods are firm and uncluttered, and the recent rush of mathematical models receives sober treatment. The theme of the book reaches from simple solutions of amphipathic molecules to the structural basics of biomembranes, and in so slim a volume it would be difficult to please all readers with the choice of topics and facts. The omissions are almost as interesting as the inclusions. For example, bile salts are given short shrift, and the relation of dilute micellar solutions to the full phase diagrams is only touched upon. Wilkins *et al.* are quoted in support of the lipid bilayer model for erythrocytes, but Blaurock's reconsideration is not. Given that biomembranes are the sites of extensive chemical activity, the lack of reference to the now substantial body of work on micellar catalysis is a pity.

In the account of biomembranes it is good to see Robertson getting proper credit for his contribution to the bilayer membrane hypothesis. The now conventional view that biomembranes are fluid mosaics based on lipid bilayers is the one adopted by Dr Tanford, and to me the important last chapter (on membrane proteins) consequently has the air of skilful fitting of the hydrophobic proteins to a Procrustean bed. Perhaps when physicochemical data on the proteins are as extensive as for the lipids, the remarks of J.B.S. Haldane (quoted at the front of the book), which identify the membrane polymers as the key components, will be seen as truly prescient and the role of the lipids will be put into better perspective. Be that as it may, the hydrophobic effect will remain of major importance in the study of lipids and proteins alike, and this book will beneficially influence future research on membranes and other structures. We should hope for a third edition for the pleasure of reading Dr Tanford again. □

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